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VESTIGIAL CHARACTERS OF TERMITES AND PROCESSES OF REGRESSIVE EVOLUTION[1]

ALFRED E. EMERSON

Department of Zoology, The University of Chicago

Received January 27, 1961

Great strides have been made during the last century in the elucidation and analysis of processes of organic evolution. The modern synthetic theory of evolution rests upon a wealth of data accumulated by means of many techniques applied to many biological phenomena, particularly from the sciences of comparative morphology, paleontology, systematics, biogeography, ecology, and genetics. Comparative biochemistry, physiology, embryology, and behavior are at the threshold of rapid advances, and are beginning to provide significant information concerning evolutionary events and processes. However, the evolutionary interpretations from one discipline do not always seem to be consistent with those from others; so we are confronted with differences of opinion among competent scientists, inadequate integration of portions of the time-dimensional pattern, and serious rifts in the complicated matrix of related data from which our knowledge develops. It is my purpose here to consider a few facts, try to interpret these facts in the light of modern biology, and attempt to resolve some current evolutionary problems.

One often gains perspective by looking at the reverse of an image. Much attention has been paid to the processes of genetic change by means of mutation and recombination, but it is constructive to compare evolutionary change to genetic stability, replication, and the persistence of complex genetic systems over long periods of time. Emphasis has been placed upon evolutionary advance by means of natural selection, but for contrast it may be well to consider the regression of former adaptations and the occurrence of non-adaptive vestiges (see Van Valen, 1960). It is expected that the complementarity of the positive and negative aspects of evolution may aid us in understanding evolutionary processes as a whole.

TERMITE EVOLUTION

Most of the factual information used for this discussion is drawn from the study of termites, an order of insects to which I have devoted a lifetime. Pertinent detailed data are available from the publications of numerous investigators in many countries. Here I shall confine myself to a few illustrative examples and a brief summary of some theoretical implications.

No single organism or group of related organisms can provide sufficient data for the analysis of all evolutionary processes.

[1] Presidential Address, Society for the Study of Evolution, Fifteenth Annual Meeting, New York, December, 1960.

Analytical studies in some important fields of research have not been made on termites. (1) Genetic mechanisms have not been investigated directly in these social insects. (2) Biochemical studies of termites are negligible. (3) Physiological researches are few, although some interesting information is available. (4) Embryological and developmental processes have not been studied to much extent, although we have some important experiments on caste determination. (5) The number of well-preserved fossils is few (about 75 fossil species, mainly from temperate Tertiary deposits, are known and less than half are well enough preserved to allow accurate phylogenetic placement). (6) The evidence of evolutionary change is almost wholly circumstantial. (7) Most of our information is based upon observations and statistical analyses rather than controlled experiments.

We have to assume that the general principles of biology derived from researches on more appropriate organisms (Muller, 1950; Dobzhansky, 1959) are applicable by analogy to termites. For example, we may assume that complex genetic patterns never originate twice or, if lost, never appear again in evolutionary history.

In spite of the recognized limitations of termites for certain types of investigation, these insects offer excellent material for understanding some important evolutionary processes. (1) They exhibit a social division of labor within the same colony between sharply distinct castes, some of which are unable to reproduce, and each of which owes its post-embryonic development (with the exception of genetic sex determination) to physiological rather than to genetic differences. (2) Each caste has known physiological, developmental, structural, behavioral, and ecological distinctions, and all types are found among the progeny of a single pair of parents. (3) The interdependent individuals are aggregated into a population system that shows striking functional analogy to the integrated cellular or multicellular individual organism. This supraorganismic system must be an evolutionary unit with emergent properties transcending the summation of the attributes of the included individuals. (4) The taxonomy, particularly at the generic level, is in some respects based upon sounder objective evidence than has been gathered for any other comparable invertebrate or vertebrate group of animals with a similar number of genera and species (about 190 genera and 2100 species have been studied). (5) About 30 fossil species are sufficiently well preserved to give direct evidence of the existence of some fairly advanced living genera in Eocene times, and also to provide extinct connecting links between a few modern genera. (6) The taxonomic relations of modern genera are amply substantiated by constellations of characters. (7) The numerous gradations which connect taxa give evidence that termites as a whole are bradytelic. Numerous genera ancestral to living genera are still in existence. In other words, many "relicts" or "living fossils" exist at present. (8) The correlations between the geographical distribution of the genera and their taxonomic classification are remarkable, and these enable us to determine origins in time and place, and directions and periods of dispersion. The wealth of circumstantial evidence not only enables us to detect evolutionary origins during Tertiary times, but also Mesozoic origins and dispersions of many living genera, subfamilies, and families, despite the absence of Mesozoic fossils. (9) Ecological adjustments, tolerations, limitations, and distributions are sufficiently known to provide significant data on physiological, structural, and behavioral adaptations. (10) The supra-organismic integration of individuals by means of stimulus and response across non-living space substantiates concepts of the evolution of behavior which are analogous to the evolution of biochemical and biophysical integrative mechanisms in the individual organism with protoplasmic contiguity. (11) The nest-building behavior of some termites results in distinctive specific and generic structures that exhibit many principles founded on morphol-

ogy, development, physiology, genetics, taxonomy, and phylogeny. Behavior may be appropriately referred to under such terms as taxa, homology, analogy, symmetry, replication, metamerism, regeneration, fields, gradients, recapitulation, genetic assimilation, vestiges, and homeostasis. These significant analogies are strongly indicative of common evolutionary processes determining both behavior and structure. (12) The social adaptations of termites enable them to reduce environmental fluctuations and to control physical and biotic factors at optimal levels (social homeostasis). (13) Many other organisms, both plant and animal, partake of the individual and social homeostasis of termites, and indicate a reciprocal phylogeny from Mesozoic times to the present. Examples are found among taxa of flagellate protozoans and staphylinid beetles (also see Buchner, 1960). (14) Much evidence supports the notion of a definable ecosystem that incorporates and adaptively modifies the physical and biotic environment through long evolutionary time.

All comparative characters of termites (or any other organisms) are by no means equally valuable for making phylogenetic judgments (Schmidt and Emerson, 1960). Relationships and gradations often occur between conceptual dichotomies, and these should be carefully evaluated. However it is often possible to distinguish and compare homology to analogy and both to fortuitous resemblance, strongly adaptive functional characters to weakly adaptive or nonfunctional characters, primitive to derivative characters, generalized to specialized characters, rapidly evolving to slowly evolving characters, persistent stable characters to labile changing characters, qualitative to quantitative characters, progressively evolved characters to regressed vestiges, relatively complex to relatively simple characters, characters of developmental stages to adult characters, monomorphic to polymorphic characters, individually acquired characters to hereditary characters, constellations of associated characters to single characters, and individual variation to variation of colony populations, demes, subspecies, species, genera, and more inclusive taxa.

By the assemblage of multiple bits of evidence and by making careful comparisons, evaluations, and interpretations, we are often able to show order where the relations of the facts have been obscure, to substantiate one hypothesis to the exclusion of others, and to make predictions of future discoveries based upon verified evolutionary concepts and directional trends. Trends may be grouped under positive progressions, negative regressions, and those that progress in some taxa and regress in others (Emerson MS). Evolutionary advances are most often associated with adaptations that facilitate intraspecies social functions and adjustments of the species to the ecological environment, particularly defenses against predatory enemies. The evolutionary regressions are more numerous than the detected advances, and often seem to be reductions associated with adaptive advances in other directions. In particular, endogenous adaptations of the individual regress as it becomes more highly adapted to an interdependent society, and the intraspecific adaptations of a species population may regress as it becomes more highly adapted to an ecological community or ecosystem.

Evolution of Adaptation

A consideration of numerous phylogenetic advances and regressions in termites allows certain somewhat tentative generalizations. A few of the advances are improved adjustment to types of food (grass, humus, fungus) compared to earlier restriction to decayed or hard wood. A conspicuous advance is seen in the defensive mechanisms of the soldiers against predatory enemies. But most of the adaptive trends seem to indicate an evolutionary increase of numbers of individuals in a colony, increase of social division of labor, and increase in social integration. According to the generally accepted theory of nat-

ural selection, we may expect to find selective pressures that quantitatively affect the survival of favorable genetic variations and the elimination of unfavorable variations. Selection guides and channelizes evolutionary changes. I see no reason to suppose that an increased functional specialization of individuals in an insect society, or an increased cooperation between individuals, is a directional trend unless division of labor and integration together have a selective value.

The concept that seems to have the most substantiating evidence is that natural selection guides termite evolution toward improved homeostasis. Homeostasis is a term coined by Cannon (1932) for the regulation of optimal conditions of biological existence, both maintenance and reproduction. He was fully aware that stable equilibrium was not always optimal, but that disequilibria were often functional and were maintained by separation in space (structure) or separation in time (endogenous periodicity and rhythmicity) (see Waddington, 1957, p. 32). Following the lead of Claude Bernard, Cannon concentrated on physiological homeostasis within the individual organism and the regulatory feedback mechanisms. Others (Lerner, 1954) have concentrated on genetic homeostasis. Cannon applied the concept to human society with its behavioral division of labor and integration by means of learned symbolic communication. (In a very good and thought-provoking book that synthesizes genetics, development, and evolution applicable to man, Medawar, 1960, p. 99, says that the idea that "division of labour within a [human] society is akin to what we can see in colonies of insects" is a bad judgment based upon bad biology. Emerson, 1954, p. 72, thinks otherwise.) Selection pressures on genetic variables affecting genetic and physiological homeostasis of individuals surely channelizes evolutionary directions. The evolutionary trends toward social homeostasis in insects and men, although analogous, result in comparable functions. Selection resulting from non-genetic learning through experience guides the genetic evolution of mimicry in butterflies and the genetic evolution of domestic varieties of plants and animals. The phenotypic ability to learn in animals and man depends in part upon genotypic attributes that presumably appear during the course of organic evolution. Improved homeostasis seems to be a universal evolutionary trend and is basic to the concepts of adaptation and adaptability applied to all levels of biological and cultural organization. This sweeping generalization of course should be tested and retested during the evolution of science—an important aspect of cultural evolution.

FIG. 1. Nasute soldier of *Nasutitermes guayanae* (Holmgren) with vestigial mandibles.

CORRELATED PROGRESSIVE AND REGRESSIVE EVOLUTION

Termites provide excellent illustrations of the association of advancing adaptive evolution with regression of earlier functions. Possibly the best example is found in the subfamily Nasutitermitinae (Termitidae), which includes many of the most advanced termite genera. The derivative genera in this subfamily are characterized by the nasute soldier—a soldier with a prolonged portion of the head (fig. 1) that

FIG. 2. Mandibulate soldier of *Armitermes neotenicus* Holmgren, a transitional genus between the primitive mandibulate soldier with a small "squirt gun" and the nasute soldier with vestigial mandibles.

serves for the forcible ejection of a sticky and irritating fluid onto its enemies, particularly ants. Along with the specialization of the "squirt gun" apparatus, the nasute soldier has vestigial functionless mandibles. A sufficient number of species and genera exist so that we can be confident that the primitive and transitional genera in the same subfamily, which possessed soldiers with large functional biting mandibles and the "squirt gun" protective adaptation (fig. 2), gave rise to the genera with nasute soldiers, and that this was a one-way evolutionary directional trend. Eight relatively primitive genera with mandibulate soldiers occur only in the Neotropical region, while genera with nasute soldiers occur in all tropical regions over the earth (fig. 4). Some experimental evidence on the relative defense abilities of various mandibulate and nasute soldiers in this sequence against one species of predatory ant indicates the evolution of improved protective adaptations and the loss of former protective adaptations (table 1). Some highly advanced genera have distinctive dimorphic or trimorphic soldiers, each soldier type probably with somewhat different protective functions. The imago-worker mandibles of these same genera retain their original wood or leaf-cutting and chewing functions with little indication of change during the obvious adaptive advance of the soldier caste. The imago-worker mandibles are very conservative and often show no marked change in the sequences of allied genera (Ahmad, 1950). However, some show slight changes in the inner cutting margin of the left mandible and in the proportional size

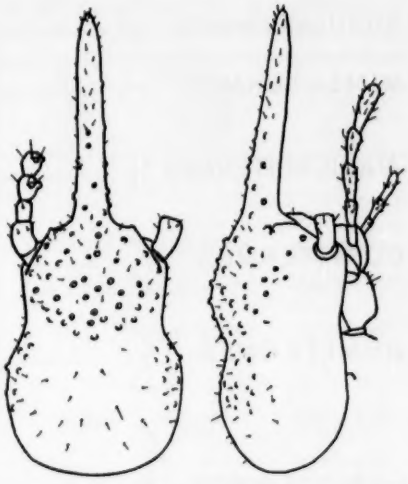

FIG. 3. Top and side of head of nasute soldier of *Verrucositermes tuberosus* Emerson, an African genus on the *Subulitermes*-branch of the Nasutitermitinae with vestigial mandibles that lack both marginal teeth and apical points.

of the apical and marginal teeth. It is very difficult to assign adaptive function to these small evolutionary changes in the structure of the imago-worker mandible, but it is quite clear that groups of related genera can be arranged in a phylogenetic tree, particularly when numerous other associated characters of all castes are considered. The indicated phylogenetic order correlates well with orderly sequences of geographical distribution (Emerson, 1955), ecological adjustment, and behavior. The taxonomy and phylogeny of 60 genera and 159 species of termitophilous staphylinid beetles found only in the nests of the Nasutitermitinae correlate remarkably well with that of their hosts (Seevers, 1957, 1960). By means of these constellations of associated characters, we are able to make the highly probable inference that the origin of the nasute soldier from more primitive mandibulate soldiers was diphyletic (fig. 3). We may conclude that the protective adaptation of the nasute soldier together with its correlated regression of the mandibles occurred independently in two branches of the phy-

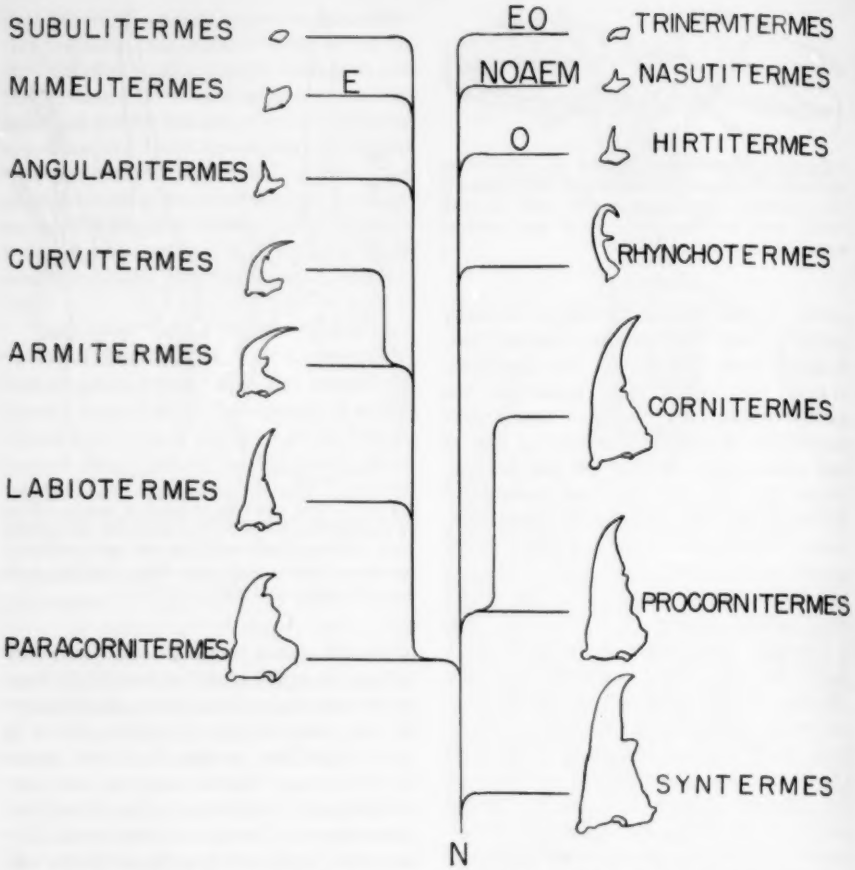

FIG. 4. Parallel diphyletic regressive evolution of the soldier mandible in the Nasutitermitinae. N = Neotropical. O = Oriental. A = Australian. E = Ethiopian. M = Malagasy.

logenetic tree (fig. 4). In other words, the nasute soldiers are diphyletic, convergent, and analogous, in spite of the fact that both branches evolved from primitive genera possessing mandibulate soldiers.

Despite the analogies, the basic primitive stock possessed soldiers with homologous small "squirt guns" and defensive biting mandibles in addition to homologous imago-worker mandibles. Several of these primitive South American genera still exist relatively little changed since Cretaceous times (Emerson, 1945, 1955). Such independent progressive and regressive evolution of homologous organs has been called *parallel evolution*. The independently evolved progressive specialization of the "squirt gun" of the nasute soldier may be ascribed to *orthoselection*. We need a theory in conformity with sound biological principles to account for the parallel regression of the nasute soldier mandibles.

It is possible to illustrate certain concepts in arranging the facts of the evolution of the Nasutitermitinae. The origin of the sterile castes of termites clearly indicates *caenogenetic* evolution. A soldier is a specialized nymphal stage and not an adult

TABLE 1. *Summary of experiments on the defensive ability of certain termites in two branches of Nasutitermitinae against the predation by the ant,* Paratrachina longicornis

	Workers		Soldiers			
	No.	Av. ants repelled by bite ± SE	No.	Av. ants repelled by bite ± SE	Av. ants repelled by gland ± SE	Total ants repelled ± SE
Nasutitermes — Branch						
Cornitermes walkeri	79	0.06 ± 0.03	79	1.58 ± 0.15	0.58 ± 0.14	2.16 ± 0.22
Rhynchotermes perarmatus	72	0.03 ± 0.02	101	0.04 ± 0.02	5.58 ± 0.78	5.62 ± 0.78
Nasutitermes columbicus	–	–	108	0	5.63 ± 0.45	5.63 ± 0.45
Subulitermes — Branch						
Armitermes chagresi	104	0.02 ± 0.01	168	0.15 ± 0.03	6.50 ± 0.64	6.65 ± 0.67
Subulitermes zeteki	88	0.01 ± 0.01	88	0	8.31 ± 0.56	8.31 ± 0.56

stage of its non-social ancestors. However, the nasute soldier has become a non-molting and non-reproducing adult. The further evolution of the adult soldier toward improved protective adaptations that are not manifest in the reproductive castes may be called *deuterogenetic*. Deuterogenesis is even more clearly exhibited in the evolution of specialized function of the post-pupal adult sterile castes of ants and other social Hymenoptera. The developing nasute soldier also indicates *palingenesis* and *recapitulation*—concepts which will be referred to later. *Teleonomy* or end-directed evolution (Pittendrigh, 1958) is manifest. Adaptations to future functions are possible when a feedback from effects to repeated causes occurs. Natural selection provides such feedbacks and integrates evolutionary time within the multidimensional social system.

Can the concepts of adaptation and improved homeostasis be applied to both progressive and regressive evolution of nasute soldiers? Adaptation (both general and special) is usually considered to be a somewhat larger concept than homeostasis because adaptation involves fitness to environmental conditions, both physical and biotic. But fitness to an external habitat may ease the problems of internal homeostasis so that variations affecting both interacting exoadaptation and endoadaptation may be selected for their combined functional values and reciprocal balance. The social homeostasis of termites regulates the individual environment by bringing such factors as humidity, food, and predatory attack toward optimal values (Emerson, 1956). With the improvement of social homeostasis, the necessity of individual adaptation to a less regulated environment is reduced. For example, the known facts support the theory that the structures and processes that served the less social ancestral cockroach in its more variable habitat could and did regress as the regulation of more optimal external conditions of the individual termite increased through social division of labor and social integration. This theory seems to provide a partial explanation for the regressive trends in the evolution of termites that are compensated by the progressive evolutionary trends of social homeostasis. By a similar type of reasoning, we may come to some understanding of the regressive evolution of the mouthparts of some termitophilous beetles as they adaptively evolve dependence upon the social homeostasis of their hosts. It

seems to me that this theory avoids mystical explanations of adaptation inappropriate to science (see Lack, 1960).

Regressive Evolution of Termites

It has often been stated that structures undergoing evolutionary reduction are more variable than those with important functions. The third molars of man afford an example. In the light of modern genetics, we may assume that an important function is maintained by strong positive selection pressures and that variations that affect such a function may be subject to negative selection pressures (Muller, 1950, p. 214). Relative stability of a functional structure is thus maintained. Variability of a part with a reduced function, however, might be tolerated without drastic elimination (see Breder, 1944).

Variation of one character may be correlated in development with another. Huxley (1932) gives many instances of allometric (heterogonic) growth relations. De Beer (1958a, p. 92) points out that if an animal with an allometric organ were to decrease the size of its body, that organ would become vestigial and would finally disappear. Darwin had no satisfactory answer for further evolutionary reduction after a vestige had lost its function. Allometry provides one possible answer that fits some cases but not all.

Among termites, a good example of evolutionary reduction is found in the nasute soldier mandibles (fig. 4) of certain species (Emerson, 1960a, p. 7). Some soldiers have vestigial functionless mandibles with the apical points still present. The mandibles of other soldiers lack apical points, sometimes only on one side of the same individual. Several genera include some species whose soldiers have points and other species without them. In most cases, however, the presence or absence of apical points, and even slight quantitative differences in proportions, are generically stable.

The observed variability within species, between species of the same genus, and between genera might be due to gene pen-

Fig. 5. Mandibles of ergatoid nymph of *Archotermopsis wroughtoni* (Desneux), the most primitive mandibles among living termites. Arrow points to the subsidiary tooth.

etrance at different physiological thresholds, or it might be due to an inhibitory effect of one or numerous alleles or other genes. We do not know what the precise mechanisms are, but we do know that variation in the same individual or in different castes within the same colony of nearly all social insects most probably is due to physiological thresholds and not to genetic differences. We also know that subspecies variation or variation between closely allied species of animals is only rarely the result of relatively simple genetic differences. Adaptive and taxonomic characters are almost always the result of complex coadapted multiple gene systems.

In contrast to the stability of gradations in functionless vestiges, we also have astonishing stability over many millions of years in some structures that seemingly never had much if any function. An example is what I have called the subsidiary tooth of the right imago-worker mandible of certain termites. This tiny hump or minute projection is present at the base of the forward edge of the first marginal tooth in several relict genera including the most primitive living genus, *Archotermopsis* (fig. 5), and the archaic genus *Stolotermes* (Emerson, 1933, 1942; Ahmad, 1950). It is characteristic of all genera of the moderately advanced family Rhinotermitidae, and it is seen in the most primitive genera of several subfamilies of the advanced

FIG. 6. Mandibles of imago of *Protohamitermes globiceps* Holmgren, the most primitive mandibles of the basic living subfamily Amitermitinae of the most advanced family Termitidae. Arrow points to the subsidiary tooth.

family Termitidae (fig. 6). The subsidiary tooth is reduced or absent in the clearly derivative mandibles of certain advanced families and genera (including the relict *Mastotermes*, all of the Kalotermitidae, the Hodotermitinae and the Nasutitermitinae). The taxonomic distribution of the subsidiary tooth among primitive termites indicates a most remarkable evolutionary stability with little variation except for the gradual and often parallel reduction and elimination in derivative groups. There is no evidence that indicates an adaptive function, and some circumstantial evidence that indicates little if any function. However, the character has probably been present throughout the major adaptive radiations of termites that started in early Mesozoic times (Emerson, 1955). Several fossil species from Eocene and Oligocene strata provide direct evidence of the existence of this structure for at least 50 million years.

RECAPITULATION OF VESTIGES

Possibly the best example of recapitulation (an aspect of *palingenesis*) among termites is found in the developing soldier mandible of several genera (figs. 7, 8, 9). Hare (1937) figures the developing soldier mandible of *Nasutitermes*, *Microcerotermes*, and *Termes* (Termitidae), each of which lacks a marginal tooth in the adult soldier, but has a distinct tooth in the middle of the inside edge of the soldier nymph mandible. The phylogenetic arrangement of these genera as shown by Holmgren (1912), Hare (1937), and Ahmad (1950) indicates that marginal mandibular teeth with a defensive function were present in the ancestral soldier types represented by the primitive living genera *Syntermes* (Nasutitermitinae), *Eurytermes* (Amitermitinae), and *Hoplognathotermes* (Termitinae) of their respective subfamilies of the most advanced family Termitidae.

It is important to remember that the recapitulative characters of the teeth in these soldier nymphs are associated with phylogenetically conservative and closely similar imago-worker mandibles in related genera. The soldier nymph is soft-bodied and has no immediate social function, although it surely has a function in development and trophallactic social exchange. The worker-like nymph preceding the soldier nymph sometimes has a social function closely similar to that of the adult worker. The original biting and chewing function of the imago-worker mandibles shows no known adaptive radiation associated with changes of type of food from wood to leaves or humus. In contrast, the adult soldier mandibles (fig. 10) exhibit marked adaptive radiation of defensive functions only. The genetic system basic to the divergent epigenetic expression of the imago-worker type of mandible and the soldier mandible must be essentially identical in the same individual during its developmental stages, and is presumably nearly as identical in the different adult castes of the same colony. The homologous mandibles in different castes show both phylogenetic stability and striking adaptive changes at the same time. They are both relatively bradytelic and tachytelic (Simpson, 1944).

THEORY OF REGRESSIVE EVOLUTION

With the advances in modern genetics, physiological genetics, and developmental biology (Dobzhansky, 1959; Anfinsen,

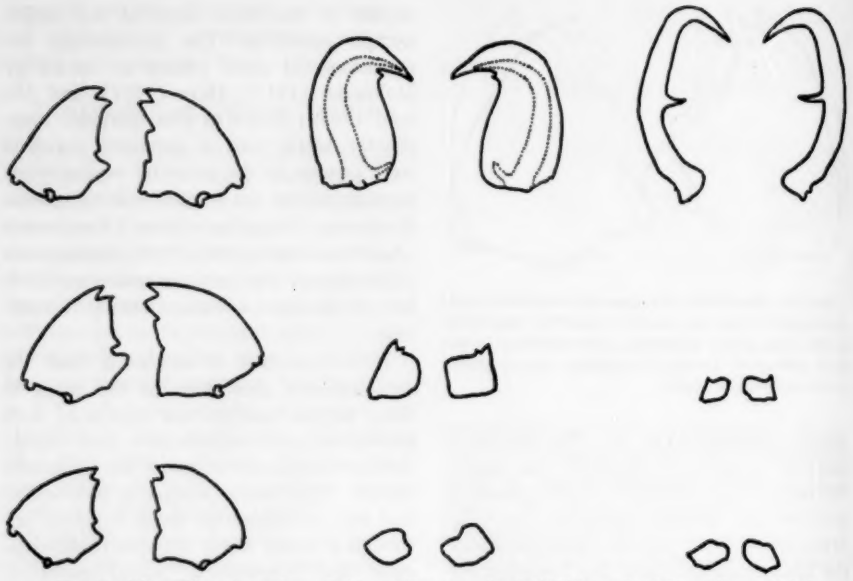

FIG. 7. Mandibles of workers (left), soldier nymphs (middle), and adult soldiers (right) in the Nasutitermitinae. Top row—*Rhynchotermes perarmatus* Snyder with functional soldier mandibles and marginal teeth. Middle row—*Nasutitermes nigriceps* (Haldemann) with non-functional soldier mandibles with apical points and recapitulated marginal teeth in the soldier nymph. Bottom row—*Trinervitermes brutus* (Sjöstedt), an advanced genus without points or marginal teeth in the soldier nymph or adult.

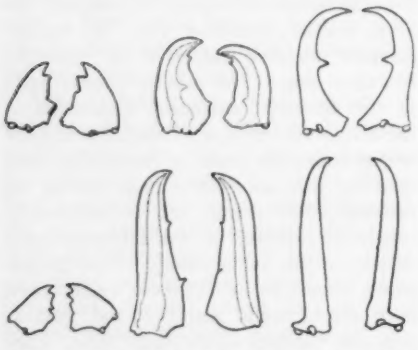

FIG. 8. Mandibles of workers (left), soldier nymphs (middle), and adult soldiers (right) in the Amitermitinae. Top row—*Amitermes foreli* Wasmann, a genus with marginal teeth in the biting soldier mandibles. Bottom row—*Microcerotermes arboreus* Emerson, an advanced genus often without marginal teeth in the biting mandible of the adult soldier, but with recapitulated teeth in the soldier nymph.

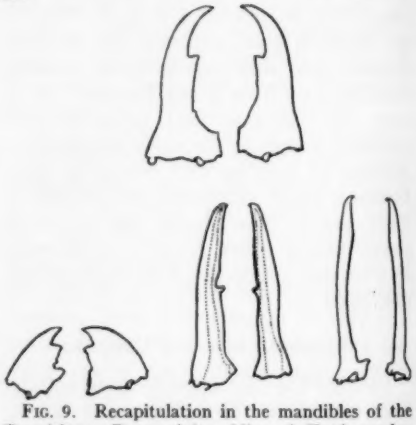

FIG. 9. Recapitulation in the mandibles of the Termitinae. Top—adult soldier of *Hoplognathotermes subterraneus* Silvestri, a primitive genus with biting mandibles and marginal teeth. Bottom row—worker (left), soldier nymph (middle), and adult soldier (right) of *Termes panamaensis* (Snyder), an advanced genus with defensive symmetrical snapping mandibles with recapitulated functionless marginal teeth in the soldier nymph mandibles.

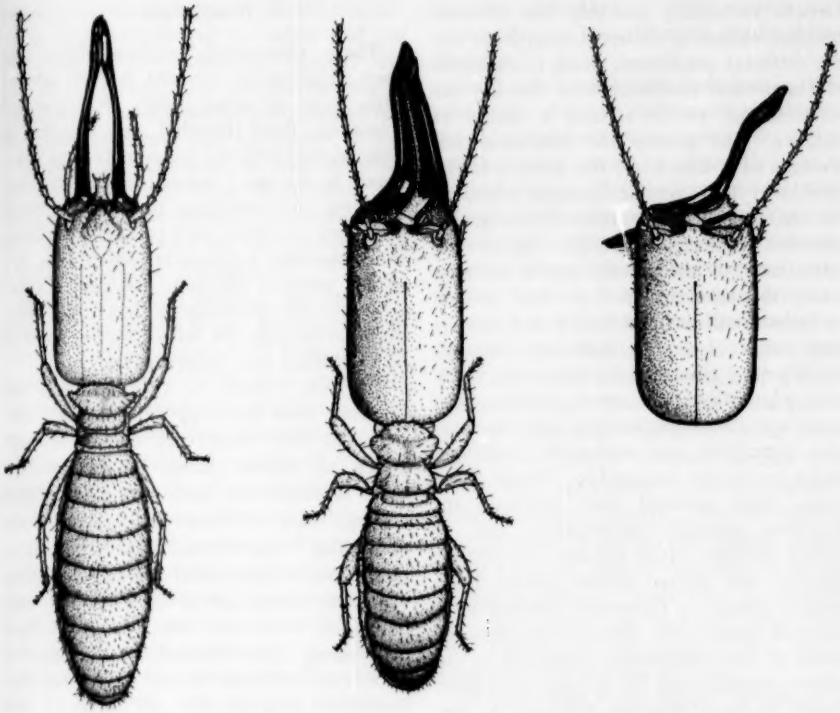

FIG. 10. Soldiers of advanced genera of Termitinae with non-biting relatively symmetrical snapping mandibles (left) and derivative asymmetrical snapping mandibles (middle and right). Left—*Termes mediocula tus* Emerson in resting position. Middle and right—*Neocapritermes angusticeps* Emerson in resting and snapped position.

1959; Nanney, 1960), together with more abundant information about physiological, structural, behavioral, and ecological functions, we are in a position to outline the major factors that influence regressive evolution and the retention of vestiges of former adaptations. Drawing largely from the ideas expressed by Wright (1929, 1934a, 1934b) and reconsidering discussions by Fisher (1930), Needham (1930), and Breder (1944), I attempted to formulate a theory of regressive evolution over a decade ago (Allee, Emerson, Park, Park, and Schmidt, 1949, pp. 635–637, 672–679). With modifications suggested by recent investigations, particularly from the currently active fields of biochemical and physiological genetics, we may now outline the essential aspects of the theory.

Genes and gene systems have an enormous number of diverse effects upon organisms and population systems. Adaptive characters (with very few exceptions) are initiated and modified by multiple genes. Genetic variability through either mutations or recombinations, although most often the result of chance, is nevertheless statistically predictable, and is probably an adaptive attribute of genetic systems. Adaptive mechanisms of recombination (i. e., sex) certainly evolve. Whether mechanisms of mutation adaptively evolve is a more controversial question, but an affirmative answer is credible—for example from our knowledge of mutator genes that modify the mutation rate of other genes.

Genetic variability probably has different optimal values in different organisms under different conditions. Both mechanisms of change and mechanisms of stability are selected and evolve toward a degree of balance. The greater the functional advantage of a character, the greater is the likelihood that genetic changes affecting the character will be subjected to negative selection pressures. The higher the level of adjustment to a relatively stable environment, the more likely it is that genetic variations will be eliminated and that a high order of genetic homology through evolutionary time will be attained. Complex adaptations are never perfect — too many correlated mechanisms and too complex organismic and ecological conditions make perfection impossible. Many processes, both external and internal, are doubtless partially incompatible and in partial conflict. Much failure, compromise, balance, and limited fitness result. Each genetic change is rigorously selected over multiple generations through its advantageous or disadvantageous effects upon the whole organism and its species population within its total temporal habitat. As one adjustment loses importance and is modified, positive selection pressures on any particular locus may decrease, become neutral, or even become negative. At the same time selection pressures may be increased for allelic or pleiotropic effects, thus directing a shift toward other adaptive functions without total change of the underlying genetic system. Regressed vestiges are retained through natural selection of whole balanced organismic systems.

This theory rests upon (1) the correlations of systematic order, phylogenetic order, and ecological order; (2) indications of a high degree of genetic molecular identity as well as change within the gene systems; (3) a correlation of genetic homology with developmental, anatomical, and behavioral homology; and (4) feedbacks from effects to causes progressively and exclusively guided by natural selection during evolution.

Discussion

These theoretical interpretations and their implications are not in full accord with those of some highly distinguished scientists. Even though I may disagree to some extent with the statements that I am about to discuss, I hope it is obvious that my own interpretations rest heavily upon the work of others and particularly upon the researches and conclusions of the scientists whom I mention. Reciprocal challenge through exchange of facts and judgments stimulates the self-correcting growth of knowledge and understanding.

De Beer (1958b, p. 18) says that the evidence does not support Haeckel's theory that the "embryonic stages through which an animal passes in its development represent the successive adult stages through which its ancestor evolved" but does show "that the developmental stages of the descendant repeat the corresponding developmental stages of the ancestor." He concludes (de Beer, 1958a, p. 170) that "phylogeny is provisionally to be regarded as a series of adult forms, which are disconnected and causally unrelated to one another, each adult form being the result of an ontogeny which differs from the previous one." "Phylogeny is therefore due to modified ontogeny" and "plays no causal part in determining ontogeny."

My difference of opinion with de Beer is partly semantic. He defines some terms more rigidly than I would. Recapitulative or palingenetic structures have an ontogeny, of course, but the ancestral function may be confined to the adult, as is the case in the marginal teeth of the defensive mandibles of the primitive soldier termite, the functionless vestiges of which appear in the soldier nymph and not in the adult of certain derived genera (figs. 7, 8, 9). It is obvious to me that selection of adult adaptations, such as the wings and genitalia of insects, guides the evolution of developmental processes. Thus ontogeny must be strongly affected by phylogeny, even if we accept de Beer's rigid definition of phylogeny. In my opinion, de Beer has

made an unwarranted sharp distinction between the evolution of the adult and the evolution of developmental stages. He has failed to recognize the correlations of genetic and developmental processes affecting both ontogeny and phylogeny during evolution. In particular, he does not seem to be aware that time dimensions are incorporated into genetic, developmental, and adult functions by the evolution of multiple feedback mechanisms.

Medawar (1951) states: "In the post-reproductive period of life, the direct influence of natural selection has been reduced to zero." Pearl (1930) drew essentially the same conclusion. I believe it is obvious that selection sorts variations of function in non-reproductive castes of termites and in non-reproductive somatic cells of organisms. It even seems possible that innate death mechanisms — clearly post-reproductive—evolved by means of natural selection of homeostatic population systems. How else can one explain the evolution of regressed mouthparts of mayflies or the endocrinal disruption after spawning of different but related species of salmon (Emerson, 1960b)? Medawar has failed to comprehend the evolutionary unity of the population system which may incorporate functional non-reproductive, post-reproductive, or even dead elements. The dead fecal matter of a non-reproductive worker termite is incorporated by the worker into a nest structure that has a function long after the worker is dead. The genetic basis for such behavioral functions surely is selected and progressively evolves. The evolution of vestigial behavior may also be surmised (Emerson, 1958).

Dobzhansky (1959) explains the continued similarity of an evolving organ (i.e., the eye of a fish and the eye of a mammal), not as the result of genetic identity, but as the result of high positive selection pressures for improved function in diverse habitats. He says, "The genes have changed so many times and so much during evolution that they differ in kind — they are different genes. Their residual similarity may be solely that of DNA strands with four and only four different kinds of nucleotides."

Although I agree with Dobzhansky that selection is the sole guiding process leading toward adaptive improvement, it is my opinion that his negation of genetic identity is too extreme. His theory does not seem to explain the long persistence of functionless vestiges. If a genetic system originally serving several functions changed completely under increased selection pressures for some functions but decreased selection pressures for others, we would not expect long phylogenetic maintenance of a functionless vestige. It is natural for a geneticist to emphasize variation rather than stability of genetic systems, because most of his data are derived from those genetic units that have changed. But the replicative capacity of the genetic system is even more apparent and precise than is its capacity to change. Some bits of evidence exist from which we may infer that effective portions of gene molecules are exactly replicated for millions of years.

Zinder (1960) has shown by means of recombination that two genera and species of bacteria, *Salmonella typhimurium* and *Escherichia coli*, have the same order of genes affecting the receptors for a bacteriophage (T6), resistance to a drug (sodium azide), fermentation of lactose and arabinose, and synthesis of the amino acid leucine. However, the genes affecting galactose fermentation and the synthesis of tryptophan were not present in the same order in the two organisms. We do not know how long this presumed genetic identity has remained stable during the evolutionary diversification, nor are we sure that a genus of bacteria and a genus of termites or mammals may be scientifically compared. However, this case of genetic homology accompanying evolutionary change indicates the probability of some degree of molecular identity during phylogeny.

Hubby and Throckmorton (1960) have shown the parallel reduction of pteridine pigments (drosopterines and sepiapteridine)

in the bodies (testes) of various species of *Drosophila* arranged in a phylogenetic tree. All of the primitive species have both pigments in the body and both primitive and derived species always have the pigments in the head (eyes). Therefore the regression is probably due to polyphyletic metabolic blocks in certain portions of the body only. Phylogenetic consistency of the metabolic blocks in the body is apparent, but the genetic basis for the pigments must be present in order to be expressed in the eyes of all individuals. The quantitative variation of the pigments in various species and the physiological variation in different parts of the insects point to a polygenic initiation of the pigment metabolism. Two loci in different chromosomes are known to affect the same pigment formation in *Drosophila melanogaster* (Glassman and Mitchell, 1959), so it is possible that the molecular identity of a pteridine (isoxanthopterin) does not always rest upon genetic identity although the phylogenetic pattern of 150 species of *Drosophila* suggests a degree of genetic homology as well as some genetic change.

The high degree of identity of the insulin molecule in cattle, pigs, sheep, horses, and two whales (sperm and sei) has been demonstrated by several investigators (F. Sanger, H. Tuppy, E. O. P. Thompson, H. Brown, R. Kitai, J. I. Harris, and M. A. Naughton) and is summarized by Anfinsen (1959). The B chain (phenylalanyl) and most of the A chain (glycyl) of the insulin molecule are identical in six species belonging to three orders of mammals. However, three positions in the disulfide "loop" of the A chain differ by having either of only two amino acids in each position. Alanine and Threonine interchange in one position, Serine and Glycine in the next position, and Valine and Isoleucine in the other position. The six species showed five different arrangements out of eight possible, but the pig and the sperm whale were identical throughout. The identity of three positions in the disulphide "loop" of the insulin molecule of the pig and sperm whale is clearly due to chance and not to genetic and phylogenetic homology. It seems probable, however, that the astonishing identities of the arrangements and types of amino acids making up the major portion of this protein molecule may reflect genetic and phylogenetic homology that presumably goes back to the Paleocene ancestor of these orders of mammals. Because of the important physiological function of insulin, there may be a high selection pressure that maintains the stability of the molecule through an enormous number of generations.

Remarkable cytological homology is found in different species of the same genus of symbiotic wood-digesting flagellates (*Leptospironympha*) occurring in the hind guts of the North American roach, *Cryptocercus punctulatus*, and in several species of the archaic termite, *Stolotermes*, of Australia and New Zealand (Cleveland and Day, 1958). A high degree of complex cellular stability is indicated since the origin of termites from roaches in late Paleozoic or early Mesozoic times. These anaerobic protozoans are unable to cross-infect different species of hosts under natural conditions, and have been confined to a continuously stable habitat throughout their long evolutionary history. Evolutionary regression of meiosis and fertilization (Sonneborn, 1957) has probably deprived some of these flagellates of a major mechanism producing genetic variation. The adaptive values of the elaborate cell structures of *Leptospironympha* are largely unknown. However, it is still possible that the cytological stability may be due to selective elimination of mutations, although many allied genera of intestinal flagellates have differentiated within this habitat, both within and between host species. It is also possible that closely similar functional structures may result from different genetic systems subjected to constant orthoselection.

In my opinion, the retention of vestigial structures in termites since the Cretaceous or earlier cannot be attributed to high selec-

tion pressures resulting in drastic changes in all parts of each gene molecule. I can only conclude, contrary to Dobzhansky, that homology must rest upon molecular identity of portions of the genetic system, and that the fundamental correlation between systematics and phylogeny is due to genetic homology and not to gradually evolving functional analogy. I do not have direct biochemical knowledge that gene molecules retain a degree of identity through evolutionary time, nor does Dobzhansky have direct biochemical evidence that all identity has been lost over hundreds of millions of generations. We need biochemical data on the comparison of molecular arrangements in genes before this problem is resolved, but I am willing to hazard a prediction that both molecular identity and molecular change will be found to occur together in evolving genetic systems.

Simpson (1959, p. 413) says, "It is always necessary in a formal classification to compromise sooner or later between horizontal and vertical separation of taxa." Among several suggested alternatives, he favors the inclusion of grades (see Huxley, 1958) of organization (i.e., mammals) in one taxon (i.e., class) even though the taxon is polyphyletic and may have arisen independently two or more times from a basic taxon (i.e., therapsid reptiles). Simpson (1960, 1961) provides greater details and critical discussion of the practical and theoretical problems involved in the classification of fossil and living taxa including mammal-like reptiles and mammals.

It seems to me that independently evolved parallel polyphyletic grades must be considered to be functional analogues even though they may evolve gradually by many relatively small genetic changes directed by natural selection. Monophyletic homologues, in contrast, rest upon genetic homology in my opinion, and should be used as the conceptual principle underlying systematic classification and phylogeny.

I agree that it is necessary to make compromises between horizontal and vertical separation of taxa, but the compromise does not necessitate the substitution of polyphyletic analogies for monophyletic homologies, nor should we confuse the two in principle. For example, the analogous grade of social organization in wasps, bees, and ants, much less that of termites, should not characterize a taxonomic category. More particularly, the genera of termites with diphyletic nasute soldiers should not be placed in a single taxon that excludes the more primitive genera with mandibulate soldiers. Homologies based upon a degree of genetic identity seem to me to be the most plausible concept to account for the correlations between taxonomy and phylogeny.

Anything approaching complete information on genetic biochemistry, ontogenetic processes, phylogenetic sequences, or ancestral species will never be known. Imagination is necessary in scientific advance, but our theories can be slowly substantiated or refuted by relating accurate facts gathered from many disciplines.

Summary

The division of labor between distinct castes of termites within a supraorganismic social population correlated with the systematic arrangements of many hundreds of fossil and living species provide material for the detection of phylogenetic order and evolutionary trends. A critical evaluation of theories of evolutionary processes is attempted. Adaptive evolution seems to progress in the direction of improved individual, social, and ecological homeostasis. This trend is accompanied by the regression of former adaptations often indicated by functionless vestiges. Associated progressive and regressive evolution are compared. Parallel evolution, orthoselection, caenogenesis, deuterogenesis, palingenesis, recapitulation, and teleonomy are illustrated and discussed. Phylogenetic persistence of functionless characters for millions of years is explained on the basis of homologies resulting from a degree of molecular identity in replicating genes. A theory is presented to account for

regressive evolution of characters with decreased or no function guided by natural selection of whole systems. Controversial viewpoints concerning some of the conclusions and basic assumptions are discussed.

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DEVELOPMENTAL HOMEOSTASIS IN POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*[1]

A. O. TANTAWY

Faculty of Agriculture, University of Alexandria, Alexandria, Egypt, U.A.R.

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Among the chromosomes of *Drosophila pseudoobscura* the relative frequencies of the various gene arrangements vary with their geographical distribution. Dobzhansky (1943) reported that populations of *Drosophila pseudoobscura* which live in different habitats often differ in the relative frequencies of their inversions and that the composition of a population captured from one region may differ significantly from season to season, suggesting that in nature these karyotypes are subjected to natural selection. Similar results have been obtained by Dubinin and Tiniakov (1945) for *Drosophila funebris*.

These early findings indicate that the carriers of the various gene arrangements must possess different adaptive values in various environmental conditions. Such results have been confirmed in laboratory experiments in which populations of *Drosophila* were maintained artificially in cages under different environments. Thus, the data reported indicate that at colder or warmer temperatures populations carrying different gene arrangements do not reach equilibrium at the same frequencies. Wright and Dobzhansky (1946) and Dobzhansky (1947) have attributed such a situation to the fact that the heterozygous genotypes possess a higher adaptive value than either homozygous genotype.

After the pioneer work of Dobzhansky, many other investigators worked on the effects of several environmental conditions on quantitative traits in populations of *Drosophila* possessing various gene arrangements. For instance, Heuts (1948), Wallace (1948), da Cunha (1951), Levine (1952) and Moos (1955) working on populations of *Drosophila pseudoobscura*, Spiess and Schuellein (1956) and Spiess (1958) on populations of *Drosophila persimilis*, all agree that flies with various gene arrangements do not show the same adaptive values in different environments.

There is little evidence on the differences in component of fitness between the chromosomal types of Arrowhead (AR) and Chirichaua (CH) in populations of *Drosophila pseudoobscura* despite the extensive knowledge of the behavior of these chromosomal types in populations. The present experiments were designed to investigate the adaptive values of the two homozygous populations as well as the F_1's generation maintained under different environmental conditions. Some other crosses were carried out to study the facilitation, if any, among the various arrangements of gene combinations.

TECHNICAL PROCEDURES

Two populations of *Drosophila pseudoobscrua* homozygous for the Arrowhead and Chirichaua gene arrangements in the third chromosome kept for two years in population cages were used as basis of the present experiments. The foundation stocks were prepared by using sixteen strains homozygous for AR, and thirteen strains homozygous for CH, all derived from flies collected in the locality of Pinon Flats, Mount San Jacinto in California. For more details concerning the maintenance of these populations, i.e., No. 180 for AR and 182 for CH, and crossing for population cages, see Beardmore, Dobzhansky, and Pavlovsky (1960). All populations were kept in wooden cages at 25° C from which samples used for the present experiments were taken. At the begin-

[1] The present work was carried out during the author's tenure of the Boese Fellowship at the Department of Zoology, Columbia University, New York, N. Y., U. S. A.

ning of the experiments, cups were taken from each cage to separate virgin females of both homozygous classes and then crosses were made within and between populations. These parental crosses were maintained at temperatures similar to that of the original cages. After hatching, virgin females of AR/AR, CH/CH, and AR/CH were separated from males. Crosses within and between the first two groups were carried out to obtain the parental and the first generations. Females and males of AR/CH were crossed to obtain the second generation and heterozygous females were also backcrossed with AR/AR and CH/CH males. These six different crosses were designated as P_1, P_2, F_1, F_2, $B_{F_1P_1}$, and $B_{F_1P_2}$, respectively.

Four different temperatures, i.e., 15° C, 25° C, 27°C and a fluctuating one (designated F° C), were used. For the fluctuating temperature, populations were kept for twenty-four hour periods at 15° C, 25° C, and 27° C in succession.

Lifetime egg production: Females of AR/AR, CH/CH, and AR/CH genotypes were used to estimate the lifetime egg production at the four different temperatures. At each temperature, ninety virgin females of each genotype were taken at random and crossed with the same number of males of appropriate genotypes for other parts of the experiment. Thus, the total number of females used in the present experiment was 1,080.

At each temperature, ten females and ten males were kept in separate vials. Each vial contained a paper spoon with the usual cream-of-wheat-molasses medium with a drop of Fleischmann's yeast. Every day spoons from the total of 108 vials were removed from the vials and were replaced with new spoons holding fresh food and yeast. Eggs were counted on each spoon under a binocular microscope. To avoid crowding, spoons were changed and counted twice a day at twelve-hour intervals for the first six weeks, after which counts were made daily. Such a procedure allowed us to count eggs with great accuracy and without injuring the eggs. At the time of changing spoons, dead females were counted and discarded and the death date was recorded.

Percentage of emergence: For each of the six crosses daily egg output for only twenty females were put in food bottles. To avoid crowding in bottles, eggs were counted and cultured twice a day for the first six weeks. After hatching adults were counted and classified as to sex. Such counting of adult flies took place initially at the end of the first two days of emergence and then once every four days thereafter for warmer temperatures and once a week for the colder ones.

Body size and body weight: For each cross parents were allowed to lay eggs at 15° C, 25° C, and 27° C, i.e., crosses were kept for 24 hours under each temperature and then moved to the next temperature. Following the 27° C period the parents were returned to 15° C and the sequence was repeated four times, so that eggs from five days at each temperature were collected. Eggs were cultured, not more than 100 eggs, in each food bottle. Adults after hatching were collected and kept in fresh food bottles for the measurements of body size, i.e., wing and thorax length. Body size was measured by the method recommended by Robertson and Reeve (1952a), and used by the author previously (Tantawy, 1959).

For body weight, a microchemical 100-A Quartz Helix balance was used with which individual flies can be weighed. Flies used for body weight were kept in empty vials for eight hours after hatching and then weighed immediately. Five males and five females from each bottle for each cross were measured on each of the five days giving a total of 25 males and 25 females from each cross.

Longevity: The different crosses were maintained under each of the four temperatures, and the hatched offspring used for studying longevity. From each cross at each temperature 500 pairs of males and females were used with 50 pairs being kept per food bottle. All the flies used in this study were collected within a period of 24 hours. At 15° C the surviving adults in each bottle were transferred at weekly intervals to fresh food bottles and the number of deaths (fe-

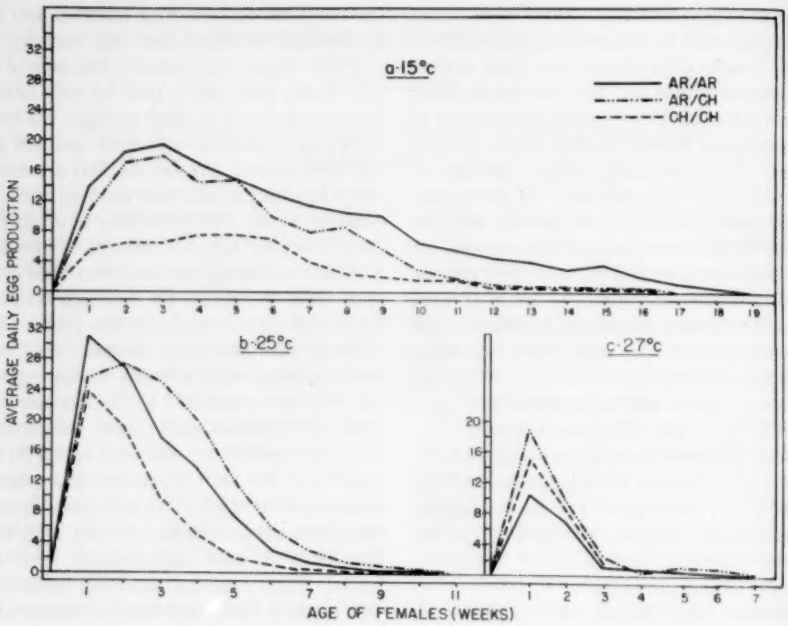

FIG. 1. Daily lifetime egg production for females of different gene arrangements at various temperatures. Each point represents the daily average in a given week.

males only) occurring during the week was determined by counting the dead flies. For warmer and fluctuating temperatures flies were transferred twice a week.

RESULTS AND DISCUSSION

Lifetime Egg Production

The relationship between lifetime egg production for females of different gene arrangements maintained at 15°C, 25°C, and 27° C are presented in figure 1. The results are reported as averages per day per female in a given week. The average daily egg production increases rapidly to the end of the first week of age in females maintained at the warmer temperatures, and more slowly at the colder one. At 25°C and 27° C the highest peaks for all genotypes are at about the end of the first week, whereas the peak appears at the end of the third week of age at 15° C. Such peaks are uniform for females with different gene arrangements (with significant differences) kept at the same temperature.

Comparing the effects of the different genotypes on egg-laying capacity, it may be seen that females of AR/AR reared at 15° C are superior to those of CH/CH, while those of AR/CH are intermediate. At 25°C the AR/CH females (apart from the first week) are superior to all other genotypes while females of AR/AR are intermediate. At both temperatures, the CH/CH females lay the fewest eggs. The situation at 27° C is different, with AR/CH females superior and AR/AR females inferior to CH/CH females.

Figure 2 reports the total lifetime egg production of the same number of females (90 females in each group) at the four different temperatures. The results show clearly significant effects of temperature on egg production which agree well with those found by Tantawy and Vetukhiv (1960) working on populations of *Drosophila pseudoob-*

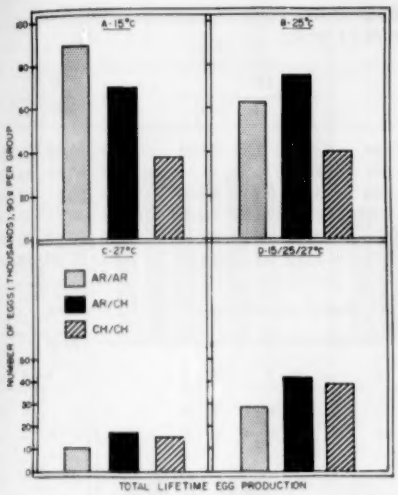

FIG. 2. Total oviposition rate for females of different genotypes at different temperatures (90 females in each group). Probabilities (based on the average lifetime per female) between:
(AR/AR–CH/CH) $<$ 0.001 at 15° C and 25° C, $<$ 0.5 at 27° C, and $<$ 0.2 at F° C.
(AR/AR–AR/CH) $<$ 0.001 at 15° C, $<$ 0.01 at 25° C and F°C, and $<$ 0.3 at 27° C.
(CH/CH–AR/CH) $<$ 0.001 at 15° C and 25° C, $<$ 0.7 at 27° C and $<$ 0.3 at F° C.

scura. The results also illustrate significant differences in egg-laying capacity between females with various gene arrangements. Tests of significance between different genotypes are presented in the caption of figure 2.

The most important feature of figure 2 is the results obtained from the fluctuating temperature. Under such temperature conditions differences in egg production between genotypes are similar to those obtained at 27° C, but the total number of eggs is intermediate between the numbers obtained at 25° C and 27° C.

The results as reported in figures 1 and 2 indicate clearly that different gene arrangements possess various adaptive temperature norms. These results will be discussed later. Similar findings have been reported earlier by Wallace (1948) who showed, in the same species, that at 25° C the SR/ST gene arrangement is superior in fecundity to the two homozygous groups and SR/SR is superior to ST/ST, but that at 16.5° C, the latter is superior to the former. Heuts (1948), Levine (1952), and Moos (1955) reported similar physiological behavior. Da Cunha (1951) obtained similar results when studying the effects of different nutritional conditions in the same species.

Percentage of Emergence and Sex-Ratio

Viability is measured as the percentage of emergence of adults from a given number of eggs cultured in ordinary food bottles. Table 1(a) reports the total lifetime egg production for twenty females in each group at each temperature and the percentage of emergence, as well as the sex-ratio. Table 1(b) shows tests of significance for percentage of emergence between different genotypes.

The results as given in table 1(a) indicate clearly significant differences between temperatures. Eggs obtained from the crosses of both homozygous groups show the highest viability at any given temperature. At 15° C and 25° C crosses within AR/AR show greater viability than those within CH/CH. But at 27° C and F° C, the latter genotype is superior to the former.

Percentage of emergence from eggs to adults is higher for the F_1 generation than for the F_2's, the differences being significant at 15° C and 25° C but not at 27° C and F° C. Females of the F_1, backcrossed to AR/AR males, show greater viability (though not significantly so) than the backcrosses to CH/CH males at 15° C and 25° C. At the other two temperatures the situation is reversed.

Sex-ratio data, as shown in table 1a(C), indicate that at 15°C and 25° C the number of females is greater though not significantly so. At 27° C and F° C there are significant differences between sexes (the level of significance is 1%) with females in excess. These results indicate that females resist changes in temperatures more, and are bet-

TABLE 1a. *Number of eggs cultured (A), percentage of emergence (B), and sex-ratio (C). See text for designation of crosses*

Crosses		15° C			25° C			27° C			F° C		
		A	B	C	A	B	C	A	B	C	A	B	C
(1)	P_1	37108	76.41	53.81	16786	59.05	53.29	2711	7.52	63.03	7624	43.88	61.01
(2)	P_2	6797	66.64	53.37	7849	52.06	57.75	5085	10.36	65.31	5693	54.61	62.45
(3)	F_1	12897	82.63	51.89	11400	66.26	54.80	3487	15.11	68.06	7202	56.11	63.15
(4)	F_2	10932	77.46	53.62	21837	56.56	56.69	3785	14.82	66.26	10834	49.27	62.21
(5)	$B_{F_1P_1}$	11983	78.67	52.96	11707	61.93	51.70	2816	12.11	64.25	4996	53.62	59.84
(6)	$B_{F_1P_2}$	7134	77.17	52.55	7040	61.72	53.20	2130	14.32	66.41	9093	56.00	57.67

b. *Probabilities for percentage of emergence (B) (based on weekly averages) between:*

Crosses	15° C						25° C						27° C						F° C					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1		*	*	–	–	–		*	*	–	–	–		–	*	*	*	*		*	*	–	*	*
2			**	**	**	**			**	*	**	**			*	*	–	*				–	*	–	–
3				*	–	*				**	–	–					–	–	–				*	–	–
4					–	–					*	*					–	–					*	*
5						–						–						–						–

* Significant at the level of 5%.
** Significant at the level of 1%.
– Insignificant.

TABLE 2. *Variance (σ^2) of logarithms of measurements for wing length, thorax length, and body weight at various temperatures. See text for designation of crosses*

Crosses	15° C		25° C		27° C	
	Males	Females	Males	Females	Males	Females
a. *Wing length*						
P_1	0.000085	0.001040	0.000067	0.000104	0.000456	0.000478
P_2	0.000314	0.000232	0.000117	0.000123	0.000332	0.000296
F_1	0.000173	0.000181	0.000109	0.000105	0.000213	0.000275
F_2	0.000364	0.000235	0.000313	0.000324	0.000378	0.000567
$B_{F_1P_1}$	0.000111	0.000205	0.000205	0.000211	0.000389	0.000453
$B_{F_1P_2}$	0.000346	0.000231	0.000318	0.000400	0.000299	0.000314
b. *Thorax length*						
P_1	0.000123	0.000215	0.000150	0.000158	0.000415	0.000462
P_2	0.000305	0.000413	0.000279	0.000341	0.000311	0.000470
F_1	0.000261	0.000197	0.000234	0.000199	0.000247	0.000234
F_2	0.000433	0.000497	0.000316	0.000405	0.000497	0.000489
$B_{F_1P_1}$	0.000364	0.000376	0.000560	0.000489	0.000539	0.001059
$B_{F_1P_2}$	0.000418	0.000395	0.000617	0.000598	0.000437	0.000493
c. *Body weight*						
P_1	0.001985	0.002531	0.000813	0.001211	0.001933	0.001598
P_2	0.002633	0.003631	0.001825	0.002031	0.001845	0.001936
F_1	0.001409	0.001788	0.001101	0.001012	0.001198	0.001937
F_2	0.002631	0.003617	0.002351	0.002616	0.001873	0.002137
$B_{F_1P_1}$	0.001636	0.002113	0.001131	0.001545	0.002714	0.002596
$B_{F_1P_2}$	0.001907	0.002231	0.001725	0.001845	0.002235	0.002411

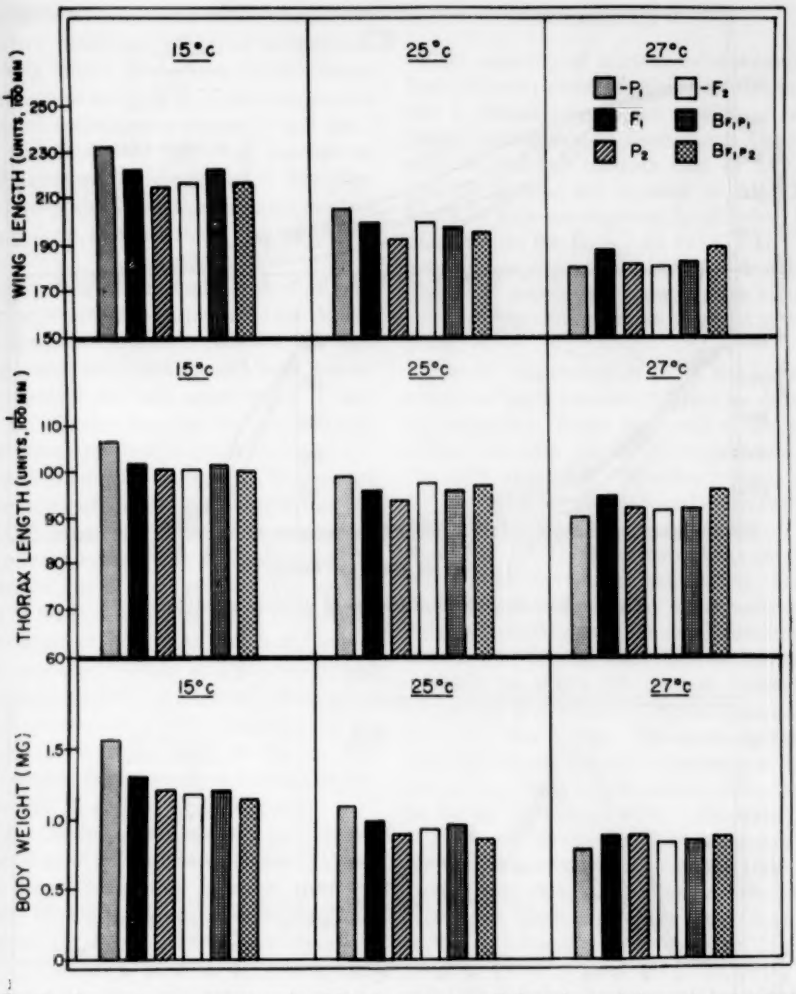

FIG. 3. Average measurements and weights (sexes averaged) for wing and thorax length 1/100 mm and body weight in mg for flies of different gene arrangements at various temperatures.

ter buffered than males. The same conclusions have been reached by Tantawy and Mallah (1961) who worked on natural populations of *D. melanogaster* and *D. simulans*.

Body Size and Body Weight

Body size, i.e., wing and thorax length, and body weight of the offspring resulting from the six different crosses are shown in figure 3, and the variance (σ^2) of logarithms of measurements are given in table 2. Logarithms have been used here to cancel out the effect of the absolute size on variance, since these are often correlated.

The results indicate highly significant differences between temperatures. The flies are much larger and heavier at colder temperatures than at warmer ones. Tantawy

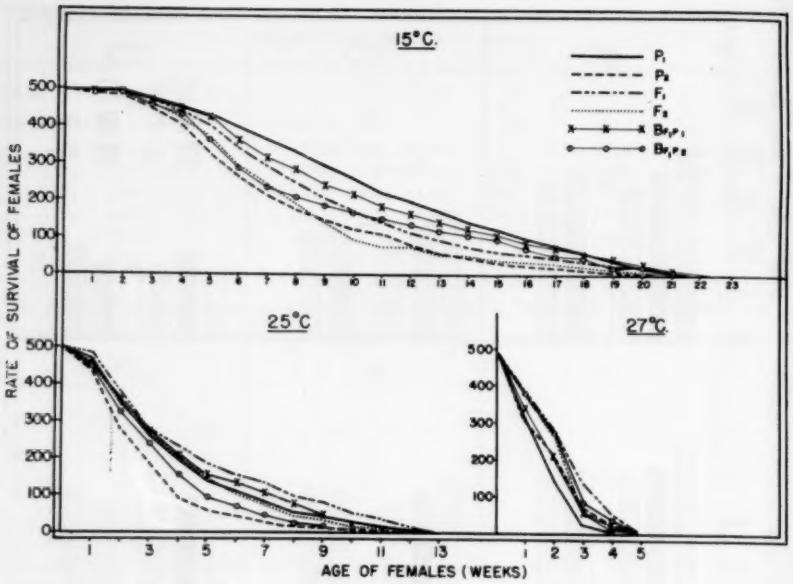

FIG. 4. Rate of survival of females of different genotypes.

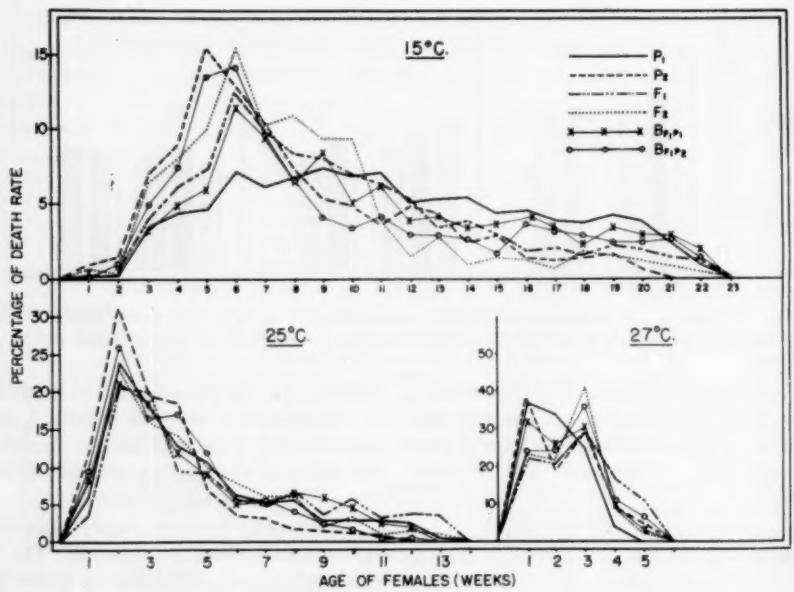

FIG. 5. Percentage of death rate in females of different genotypes.

and Mallah (1961) and Robertson (1959) reported significant effects of temperature on wing length. Robertson (1959) attributed such an increase at colder temperature "Almost entirely to a change in cell size." He also concluded that "cell number remains comparatively constant." The present results also indicate significant positive correlations between body size and body weight.

Comparing the different crosses at various temperatures, it is apparent that the offspring within the homozygous AR/AR have longer wing and thorax length with heavier body weight for both sexes at 15° C and 25° C. Crosses between the two homozygous groups produce larger offspring at 27° C, while those within the CH/CH genotype have larger offspring than those within the AR/AR group at 27° C. The excess of the F_1 generation over the mid-parent will be considered in a later section. The results also indicate a tendency for a phenotypic correlation of body size and body weight with reproductive rate, as measured by egg production.

Measurements of both characters for the F_1 generation exceed those for the F_2. The backcrosses are generally intermediate between the F_1 and the recurrent parent.

Examination of the variance (σ^2) of the logarithms of measurements (table 2) shows significant differences between temperatures. Within temperatures significant differences also occur between genotypes. At 15° C and 25° C the CH/CH genotype is more variable than AR/AR (significant at the 5% level). In all characters studied the F_1 generation is generally (but not always) less variable than parents, while the F_2 shows insignificantly higher variability than the parental generations. Offspring resulting from backcrossing F_1 females with AR/AR males at 15° C and 25° C are less variable than those obtained from CH/CH males (the differences being significant at the 5% level). The situation at 27° C is reversed in favor of CH/CH genotype.

Longevity

The longevity of adult females resulting from different crosses is shown in figures 4 and 5, respectively, as the number of surviving females and as death rate. The results in figure 4 indicate that at 15° C AR/AR females are superior to AR/CH, which in turn are superior to all other females except for $B_{F_1P_1}$. At 27° C CH/CH females live longer than AR/AR females. The F° C group (not shown) show values intermediate between those obtained at 25° C and 27° C.

Figure 5 shows that at 15° C the highest values for death rate are different for different genotypes. Peaks are found at the end of the fifth week in CH/CH females and at the sixth week of age in other groups. At 25° C the highest peak is found at the end of the second week of age in all groups. The results at 27° C, where two peaks can be found in all groups, are unexpected. They could be accounted for by some undetectable unfavorable environmental conditions occurring at the end of the third week of age.

Table 3a shows the average longevity (days) for females in the fecundity and the longevity experiments. The results indicate that females of the latter experiment live longer than those of the former. This could be due to "laboratory error." Table 3a (A, B) indicates that at 15° C the longevity of F_1 females is intermediate between those of the parents, while at the other temperatures it is higher. There is a decline (table 3a(B)) in the F_2 longevity compared with that of the F_1. At 15° C and 25° C offspring of AR/CH females backcrossed to AR/AR males live longer than those from the CH/CH backcross, while at 27° C and F° C the situation is reversed. Tests of significance (table 3b) indicate that significant differences exist between most of the longevity means at 15° C and 25° C but not at 27° C.

Conclusions

It is well known from population cage experiments with *Drosophila pseudoobscura* (Wright and Dobzhansky, 1946; and Dob-

TABLE 3a. *Average longevity (days) for females used in fecundity experiment and longevity experiment with their standard errors. See text for designation of crosses*

Crosses		15° C	25° C	27° C	F° C
A. *Fecundity experiment*					
(1)	P_1	74.18 ± 3.52	30.55 ± 3.01	11.60 ± 4.91	19.61 ± 3.81
(2)	P_2	49.93 ± 5.13	21.26 ± 4.62	14.93 ± 3.79	24.64 ± 2.91
(3)	F_1	63.92 ± 3.92	33.71 ± 2.32	16.23 ± 4.22	29.23 ± 1.38
B. *Longevity experiment*					
(4)	P_1	79.80 ± 3.01	31.46 ± 2.50	13.72 ± 4.11	20.01 ± 3.15
(5)	P_2	56.04 ± 4.21	23.24 ± 3.56	15.62 ± 4.23	26.40 ± 3.49
(6)	F_1	66.53 ± 2.34	36.85 ± 2.49	19.12 ± 3.93	30.22 ± 2.93
(7)	F_2	57.53 ± 4.39	30.73 ± 3.82	17.09 ± 4.41	24.00 ± 3.92
(8)	$B_{F_1P_1}$	74.07 ± 3.52	32.62 ± 3.44	15.68 ± 3.81	26.21 ± 3.76
(9)	$B_{F_1P_2}$	66.12 ± 4.94	27.09 ± 4.01	17.85 ± 3.34	28.05 ± 7.80

zhansky, 1948) that inversions confer different adaptive values on their carriers. Moreover, these differences in adaptive value are sensitive to temperature. Briefly, no differences in adaptive value can be observed at 15° C, while at 25° C the order of adaptive values is AR/CH>AR/AR> CH/CH. The results of the present experiments show that the components of fitness —fecundity, longevity, and viability—can in large part explain the population cage observations. In table 4 the relative fitness values of the various crosses for the different components of adaptive value are listed. The heterozygotes are arbitrarily assigned the fitness 1.000. As the table shows, the order of fitness values at 25° C is AR/CH> AR/AR>CH/CH for all components of the life cycle. At the even higher temperature of 27° C and at F°C there is still heterosis, but the fitness of the two homozygotes is reversed, i.e., AR/CH>CH/CH>AR/AR. It would be extremely informative to maintain population cages under fluctuating conditions to see whether the equilibrium frequencies of the inversion correspond to the fitness components as they do at 25° C. At 15° C the results are equivocal, with heterosis shown for viability, but not for the other fitness components. This also agrees with the population cage results, although the consistent inferiority of CH/CH at 15° C does not.

A point of considerable interest is the sim-

TABLE 3b. *Significance levels for differences in longevity: (longevity experiment). The values shown refer to % level of significance. Minus signs indicate lack of significance*

Crosses	15° C						25° C						27° C						F° C					
	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
4		6	5	5	8	7		7	8	–	–	8		–	8	–	–	8		8	8	–	8	8
5			7	–	6	8			6	8	7	–			–	–	–	–			8	–	–	–
6				7	7	–				8	8	7				–	–	–				8	8	–
7					8	8					–	–					–	–					–	–
8						8						8						–						–

(fecundity experiment)

Crosses	15° C			25° C			27° C			F° C		
	1	2	3	1	2	3	1	2	3	1	2	3
1		5	7		8	8		–	–		8	6
2			7			6			–			8

TABLE 4. *The relative fitness for flies of different gene arrangements at various temperatures*

Temperature	P_1	F_1	P_2	P_1	F_1	P_2
a. *Egg production*				c. *Longevity (fecundity experiment)*		
15° C	1.400	1.000	0.525	1.161	1.000	0.781
25° C	0.828	1.000	0.525	0.906	1.000	0.631
27° C	0.616	1.000	0.857	0.715	1.000	0.920
F° C	0.702	1.000	0.834	0.671	1.000	0.843
b. *Percentage of emergence*				*(longevity experiment)*		
15° C	0.925	1.000	0.807	1.199	1.000	0.842
25° C	0.891	1.000	0.786	0.854	1.000	0.631
27° C	0.498	1.000	0.686	0.718	1.000	0.817
F° C	0.782	1.000	0.973	0.662	1.000	0.874

ilarity of the results from the 27° C and the F° C experiments. It appears that in a fluctuating environment of high periodicity, it is the extreme high temperature that is most influential in determining results. The similarity exists not only for the fitness components but for the metric characters as well. It should be pointed out, however, that these results are at variance with those obtained by Vetukhiv and Beardmore (1959) in their experiments on crosses between geographical populations. In their work, the results under fluctuating temperature, if anything, more closely resembled those obtained under the lower temperature.

The second point of importance that can be derived from our experiments is the relation between variability and fitness. Considering variability between replicates and between individuals within temperatures, it is generally clear that greater fitness is associated with lower variability (tables 3a and 4). The superior genotype at any given temperature produces less phenotypic variability both for fitness components and for metric characters. The picture is not the same, however, when variability between temperatures is considered. For longevity (fecundity experiment) table 3a(A), the coefficient of variation of the genotypes AR/CH, AR/AR, and CH/CH are 0.517, 0.741, and 0.349, respectively. That is, the poorest genotype has the lowest coefficient of variability between temperatures. For longevity (longevity experiment), table 3a(B), the corresponding values are 0.531, 0.826, and 0.585. In this case the poorest genotype and the best show roughly equal coefficients of variability, while AR/AR individuals, whose mean longevity is not very much different from the AR/CH (36.25 days as opposed to 38.18 days), have the highest coefficient of variability. It is clear, then, that great care must be exercised in the interpretation of variability as an index of buffering.

Phenotypic variation may be caused by genetic diversity, environmental agencies, and interaction of both. Many results have been obtained to show that in populations of *Drosophila* adaptation to environment depends on a heterozygous genotype. Thus, Robertson and Reeve (1952a) and Mather (1953), and Smith and Smith (1954) have reported that, when exposed to the same variety of environmental stimuli, heterozygotes produce more uniform phenotypes than do homozygotes. The development of the heterozygotes is then on the average better buffered against environmental disturbances than that of homozygotes. Dobzhansky and Wallace (1953) considered this phenomenon to indicate a superior homeostatic adjustment of the developmental pattern in an average heterozygote. Robertson and Reeve (1952b) suggested that the decrease of the variance following hybridization may be due to greater biochemical versatility of the heterozygotes. Lewis (1954) supported the idea that biochemical versatility would be manifest, at least in part, in alternative pathways of biosynthesis functional under different environmental optima. Lerner (1954) argued that "heterozygosity has a dual function in the life of Mendelian populations. On the one hand, it provides a mechanism for maintaining genetic reserves and potential plasticity, and on the other, it permits a large proportion of individuals to exhibit combinations of phenotypic properties near the optimum. Underlying both processes is the superior buffering ability of heterozygotes with homozygotes."

TABLE 5. *Percentage of heterosis in different characters manifested at various degrees of temperature*

Character	15° C		25° C		27° C		F° C	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Egg production	3.9±1.4	—	4.8±5.6	—	35.8±4.4	—	30.2±3.9	—
Percentage of emergence	11.1±3.1	5.9±2.3	10.7±2.4	1.0±3.1	6.2±3.3	5.9±4.1	6.8±4.2	−0.1±1.4
Wing length	−0.9±0.5	−1.9±0.4	0.3±0.2	0.5±0.2	3.6±0.5	0.1±0.2	—	—
Thorax length	−2.5±0.4	−3.2±0.6	−0.5±0.4	0.9±0.5	3.7±0.3	0.7±0.4	—	—
Body weight	−8.6±2.1	−16.4±4.3	−2.0±2.4	−7.0±4.5	4.7±3.4	5.9±2.6	—	—
Longevity:								
Fecundity exp.	3.0±1.1	—	30.2±5.6	—	22.4±6.4	—	32.1±5.8	—
Longevity exp.	−2.0±2.3	−15.3±7.4	34.4±5.5	12.4±6.7	30.3±5.6	16.5±7.3	30.3±4.9	3.5±3.4

One may conclude from the above discussion that a population possesses greater adaptation than another population if it is adapted to a greater variety of environments (Lewontin, 1957). Therefore, the AR/CH heterozygotes may be viewed as being better buffered against environmental disturbances, i.e., more homeostatic, since they can live longer and produce more offspring than any other populations in a wide variety of environments. A similar test of this phenomenon was made by Dobzhansky and Levine (1955) using a range of temperature and food sources in *Drosophila pseudoobscura*. They reported that homozygotes have a low average fitness and a high variability as compared with heterozygotes.

Two explanations have been advanced to account for superior homeostasis in heterozygous individuals. The first attributes it to versatility in development of heterozygotes beyond that found in homozygotes. The second view sees no special properties in the heterozygosity except the classic one of promoting segregation, which leads to superior buffering in hybrids by virtue of the heterozygous balance achieved in outbreeding species by natural selection.

Percentage of heterosis may be expressed as the ratio of the F_1 or F_2 means to the average value for the parents (Tantawy, 1957). The results are presented in this form in table 5 which includes all characters studied at the different temperatures. The general conclusions which can be drawn from such results are that the F_1 hybrids show a heterotic increase for egg production, percentage of emergence, and longevity, over the parental level, and that the F_2 generation shows a breakdown of heterosis. Body size and body weight display negative heterosis at 15°C, and positive heterosis at 27° C. In the intermediate temperature the direction of heterosis is variable.

The results of table 5 indicate clearly that even when heterosis is present in F_1 hybrids it is not necessarily found in all characters. They are in agreement with those reported by Vetukhiv and Beardmore (1959). Another point of interest is that heterosis for most of the characters is strongest in populations reared at 27° C. It has been suggested by Lerner (1954) that euheterosis is connected with developmental homeostasis. From the results previously reported, one may gather that 27° C is the most stringent environment for populations of *Drosophila pseudoobscura*. Therefore, one expects that heterosis is likely to be more manifested at the 27° C than at other temperatures. This is in agreement with Wallace (1948), who measured many physiological traits in the life cycle of "Sex-ratio" and "Standard" genotypes of *D. pseudoobscura*. He reported that females heterozygous for ST/SR chromosomes showed heterosis more strongly at high than at low temperatures. Moriwaki, Ohnishi, and Nakajima (1956), who investigated the comparative viability of gene arrangement homozygotes and heterozygotes in *D. ananassae*, demonstrated that no heterosis was found under optimal conditions but was observed under unfavorable ones.

Summary

1. Laboratory populations of *Drosophila pseudoobscura* carrying AR/AR and CH/CH gene arrangements were the basis of the present experiments. Various crosses were made to study the productive capacity measured as lifetime egg production, percentage of emergence, body size, i.e., wing and thorax length, body weight and longevity of adult females. Four different experimental treatments were used: constant temperatures of 15° C, 25° C, 27° C, and a fluctuating one between these three.

2. Lifetime egg production differed significantly between temperatures: 15° C gave the highest egg production while 27° C gave the lowest. AR/AR was found to be the best genotype at 15° C while AR/CH is superior at 25° C, 27° C and at the fluctuating temperature. CH/CH is generally inferior to all types except that at the warmer temperatures; it is superior to AR/AR.

3. Percentage of emergence, body size, body weight and longevity give substantially the same picture as that found for egg production.

4. Variances of quantitative characters of the superior gene arrangements at a given temperature are often smaller than of the poorer ones.

5. The relative indexes of fitness show that the heterozygous genotypes are the most adapted ones, while the CH/CH is the least.

6. The F_1 hybrids generally show heterosis in egg production, viability, and longevity. Body size and body weight display more heterosis at 27° C than at 15° C or 25° C. In the F_2 generation a breakdown of heterosis occurs in all the characters studied.

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THE CONSEQUENCES OF INTERCHANGE

H. Rees

Department of Agricultural Botany, University College of Wales, Aberystwyth

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1. Introduction

It is becoming increasingly evident that both numerically and structurally the chromosome complements of different individuals within one species, even within the same population, are rarely completely uniform or constant. Investigations have shown that a chromosome polymorphism is in fact a characteristic and, what is more, an adaptive feature of many natural populations.

In respect of structural variations the most frequent causes are chromosome interchange and inversion. As a consequence of either, three genotypes may occur side by side in one population, the structural heterozygote and two classes of homozygotes—one homozygous for the old and one for the new chromosomes. Work by Dobzhanzky (1941), White (1957), and Lewis and John (1957) on inversions and interchanges that were established in natural populations of *Drosophila*, grasshoppers and cockroaches indicates that five main factors control the survival of the different chromosome types. These are their effects on the mechanics of meiosis, and hence fertility, their gene content, the background genotype associated with them, the environment, and the breeding system of the population.

The present work deals with the properties and the consequences of newly arisen structural changes in rye—interchanges not previously exposed to natural selection. The chief aim was to determine how the above genetic and environmental factors affected their behavior and survival in experimental populations.

2. Material and Method

Rye, by virtue of a self-incompatibility system, is normally outbreeding. All plants to be described are derived from a single F_1 individual obtained by crossing plants from two inbred lines, *P2* and *P8*. Two associations of four chromosomes at meiosis in the pollen mother cells showed that this F_1 individual was heterozygous for two independent interchanges. Since the parent lines and other F_1 plants obtained by crossing the lines were normal, the interchanges must have arisen spontaneously either in the gametes producing the hybrid or in the hybrid itself.

The two interchanges can be distinguished from one another at meiosis in structural heterozygotes (fig. 1). One, interchange *A*, involves chromosomes which organize the nucleolus whereas the other, interchange *B*, does not.

The history of the material bred from the F_1 is briefly as follows. The F_1 plant was self-pollinated and from its F_2 progeny three individuals, which, like the F_1, were heterozygous for both interchanges, were self-pollinated to initiate three inbred lines. Subsequent propagation of the three lines was also by self-pollination of a double heterozygote in each generation.

Progenies were grown under field conditions and, as was to be expected with forced inbreeding, the numbers surviving were low, about 47 per cent of the total in each generation. The main interest in the experiment was to determine the relative frequencies of the different interchange homozygotes and heterozygotes among the survivors. Inflorescences were therefore fixed from as many mature plants as possible for cytological classification.

3. Heterozygotes and Homozygotes

From self-pollination of the *AB* heterozygotes four cytologically recognizable classes are to be expected in the progeny. These are *Het A*, *Het B*; *Het A*, *Hom B*; *Hom A*, *Het B*; and *Hom A*, *Hom B*. In the absence of

FIG. 1. Diplotene in a pollen mother cell of a plant heterozygous for interchange *A* and interchange *B*. Ring *A* is attached to the nucleolus.

selection we should also expect to find the four classes in equal frequency. The numbers observed during the experiment are given in table 1.

The ratio for the four classes in the grand total is clearly very different from the expectation of 1:1:1:1. It follows that selection favors particular chromosome combinations at the expense of others. We need to know, from a detailed analysis of the data, which combinations they are and whether their selection varies in different lines and in different generations. For this purpose a χ^2 analysis was made of the results for generations F_3 to F_7. The complete analysis appears in table 2. The conclusions to be drawn from it are presented below.

a. *Interchange A*

Of the grand total 265 plants are heterozygous, 131 are homozygous for interchange *A*. In terms of survival the heterozygotes clearly enjoy a considerable advantage. In interchange heterozygotes the chromosomes involved in the interchange are of course heterozygous not only structurally but genically as well, particularly in rye where crossing over is mainly restricted to distal segments (see Rees, 1955). In the absence of crossing over in the main chromosome regions genic heterozygosity is consequently preserved despite the inbreeding enforced by self-pollination. Since rye normally is outbreeding and hence its genotypes normally heterozygous, it would of course be expected that heterozygous gene combinations would generally be at an advantage relative to homozygous ones. In this instance the superiority of heterozygotes, reflecting their genetic balance, is confirmed by the excess of *Het A* genotypes.

Variation between lines. From the analysis in table 2 it will be seen that there is a significant excess of *A* heterozygotes in all three lines. The ratios of *Het A* to *Hom A*, however, are not the same in all lines. The heterogeneity χ^2 between lines is 9.19 and $P=<0.05$. This variation between lines is made clear in figure 2 where the percentages of *Het A* plants among the progeny of each generation are plotted for the lines separately. It can be seen that in line 2 the advantage of *A* heterozygotes is considerably smaller than in the other two lines.

The difference in degree of heterozygous

TABLE 1. *The distributions of interchange types in the inbred lines*

	Line 1				Line 2				Line 3				Total			
	Hom A Hom B	Hom A Het B	Het A Hom B	Het A Het B	Hom A Hom B	Hom A Het B	Het A Hom B	Het A Het B	Hom A Hom B	Hom A Het B	Het A Hom B	Het A Het B	Hom A Hom B	Hom A Het B	Het A Hom B	Het A Het B
F_2													5	9	11	7
F_3	1	1	2	4	5	5	8	4	5	3	6	5	11	9	16	13
F_4	3	4	9	13	8	6	10	10	0	1	7	7	11	11	26	30
F_5	2	5	4	2	2	1	3	5	2	0	1	5	6	6	8	12
F_6	1	0	4	5	10	7	11	11	3	4	13	14	14	11	28	30
F_7	1	4	8	5	17	18	33	20	7	5	15	21	25	27	56	46
Total	8	14	27	29	42	37	65	50	17	13	42	52	67	64	134	131

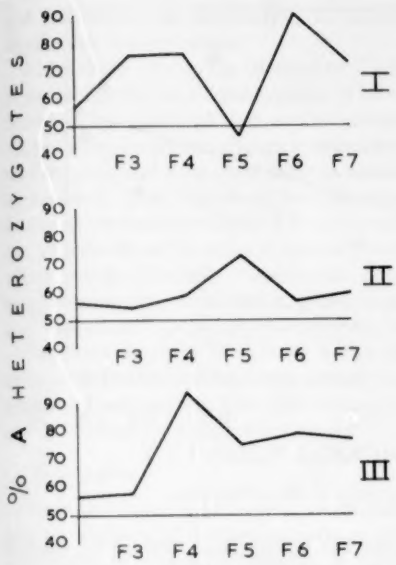

FIG. 2. Percentages of *A* heterozygotes in the three inbred lines.

advantage can of course be readily explained by genic interaction between the *A* chromosomes and the background genotype—which by segregation on inbreeding is expected to vary between the lines.

There is, however, an alternative explanation possible. A crossover, or crossovers, in a relatively proximal region in the interchange association of four chromosomes in one line would decrease the amount of genic heterozygosity in structurally heterozygous descendants. Where heterozygosity, as in this instance, is an advantage we should consequently expect the degree of advantage of structural heterozygotes in this line to diminish. While the two alternative explanations are not exclusive, evidence will be produced later to indicate that in part the variation between lines may be accounted for by this second factor.

Variation between generations. Two variables might be expected to affect the proportions of heterozygotes in different generations. The first is fluctuation in selection pressure depending upon the severity of the season. The second is the increasing homozygosity with inbreeding of chromosomes not involved by interchange. As the genotypes in general become more homozygous, and consequently less vigorous, we should expect therefore the relative advantage of the genic heterozygosity that is maintained by interchange to increase. From the χ^2 analysis we find no evidence of heterogeneity between the generations (P=0.7–0.5), and there is no evidence either that different lines react differently in the different seasons (P=0.5–0.3).

While the χ^2 test gives no indication of

TABLE 2. *The χ^2 analysis of the results from F_3 to F_7*

Class	Item	χ^2	N	P
Het A: Hom A	Grand total	45.34	1	0.001
	Heterogeneity between lines	9.19	2	0.02–0.01
	Heterogeneity between generations	2.48	4	0.7 –0.5
	Interaction between lines and generations	8.12	8	0.5 –0.3
Het B: Hom B	Grand total	0.09	1	0.8 –0.7
	Heterogeneity between lines	3.08	2	0.3 –0.2
	Heterogeneity between generations	1.56	4	0.9 –0.8
	Interaction between lines and generations	3.13	8	0.95–0.9
Hom A + Het A : Hom A + Het A / Hom B + Het B : Het B + Hom B	Grand total	0	1	1.0
	Heterogeneity between lines	2.31	2	0.5 –0.3
	Heterogeneity between generations	2.00	4	0.8 –0.7
	Interaction between lines and generations	7.59	8	0.5 –0.3

The χ^2 values are calculated for an expectation of a 1:1 distribution in each class.

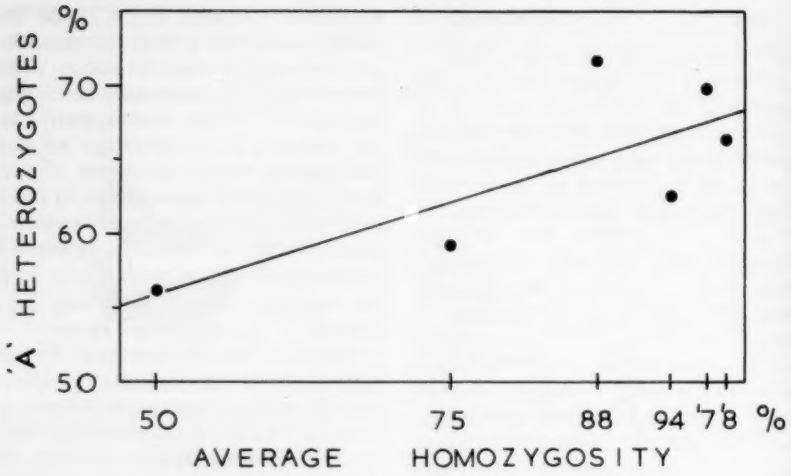

FIG. 3. Percentages of *A* heterozygotes in the inbred lines.

significant variation, a closer analysis, however, reveals a definite relation between heterozygous advantage and inbreeding. In figure 3 the percentage of *A* heterozygotes are plotted against the average degree of homozygosity for all the inbred generations, F_2 to F_7. As the homozygosity on self-pollination increases on average by a half in each generation, the horizontal scale in the graph is graduated accordingly. From this graph it is clear that the proportion of structural heterozygotes increases with more intensive inbreeding as was postulated. The probability of a positive regression occurring by chance is significant (P=0.05–0.01).

b. *Interchange B*

The superior fitness which we associate with heterozygotes in outbreeding species is attributed to a genetic balance achieved under the influence of natural selection. It does not, however, follow that every heterozygous genotype, or gene combination, is genetically balanced or even superior to the homozygotes, as was demonstrated in this material for the interchange *A*. We need therefore to inquire whether the heterozygous combination maintained by the structural change *B* displays equal superiority over the homozygotes derived by inbreeding.

For *B* the percentages of heterozygotes in the progenies are plotted for all lines in figure 4. From this and from the analysis it is immediately apparent that heterozygotes have no advantage in terms of survival over the homozygotes. Of the grand total 195 are *Het B*, 201 are *Hom B*. Ratios for lines

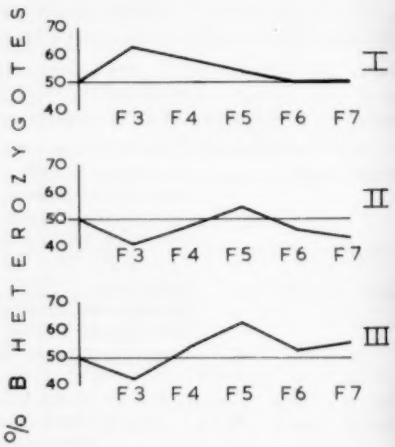

FIG. 4. Percentages of *A* heterozygotes plotted against the average homozygosity in generations F_3 to F_7.

and generations, as the analysis in table 2 shows, are homogeneous.

From these data on the survival of *A* and *B* heterozygotes and homozygotes, it must therefore be concluded that any advantage conferred by structural change is dependent on the particular gene combinations associated with it. The situation in natural populations is no doubt analogous. In particular the superiority of structural heterozygotes which results in stable chromosome polymorphism in the *Drosophila*, grasshopper and cockroach populations referred to earlier must clearly reflect choice, under the influence of natural selection, of specific and adaptive heterozygous gene complexes, and not of heterozygous complexes *per se*.

c. *Interaction*

From this experiment there is no evidence that the survival of the homozygotes or heterozygotes of either interchange is influenced differentially by the presence of homozygous and heterozygous combinations of the other. As will be seen from table 2, the x^2 for interaction between the classes is not significant; and the interchanges therefore are independent of one another both structurally and genically. In contrast White (1957) has reported a genic interaction between different structural forms of non-homologous chromosomes in wild grasshopper populations. Whether the different chromosomes interact with each other obviously again depends on their particular genic constitution.

4. Interchange Homozygotes

While it is possible to distinguish cytologically between heterozygotes and homozygotes of *A* and *B* interchanges, the two homozygous classes within each cannot be distinguished from one another by the same means. Such a distinction is desirable, particularly in *A* where there is evidence of selection, in order to gain a more complete picture of the consequences of the structural change. It is necessary to know, for example, whether the excess of heterozygotes is due to a major gene causing lethality of one of the homozygous classes. If this were so, the earlier inference of selection favoring *A* heterozygotes on the basis of their superior genetic balance would be indefensible. In two of the lines, *1* and *3*, we can, by making use of a yellow phenotype, in fact extend our investigation of the *A* interchange to include the two homozygous classes in our analysis.

The yellow phenotype. In one of the parental inbred lines, *P2*, some plants occasionally produce progeny comprising mutant yellow-leaved forms in addition to the normal green-leaved individuals. The former are small plants with relatively few tillers, fertile as female parents, sterile as males. The segregation of the two phenotypes in the F_2 and in every generation of lines *1* and *3* indicated that the yellow phenotype was controlled by a single, recessive, mutation. Evidently the original F_1 was heterozygous for the mutant allele derived from *P2*. What is more important, yellow phenotypes in lines *1* and *3* examined cytologically were found to be homozygous for interchange *A*. It follows that the gene controlling yellow is located on one of the chromosomes involved in this interchange. Also, since *all* the yellow phenotypes are homozygous for *A* and the progeny of *all A* heterozygotes in lines *1* and *3* show segregation for normal and yellow plants, there can be virtually no crossing over between the gene and the point of interchange. In other words the genetic distance between the locus and the point of interchange is very small indeed and the gene is virtually completely linked either with the normal, unchanged chromosome or with the partially homologous chromosome transformed by interchange. To determine which, a yellow, *Hom A*, plant was crossed with one from a normal rye population. At meiosis in the progeny only bivalents were found in pollen mother cells and it follows therefore that the gene (*y*) in lines *1* and *3* is located on the normal unchanged chromosome. In these two lines we are thus able to recognize all three structural classes of *A*, the heterozygote cytologi-

TABLE 3. *The distribution of interchange* A *homozygotes*

	Line 1		Line 2		Total	
	Homozygous normal	Homozygous interchange	Homozygous normal	Homozygous interchange	Homozygous normal	Homozygous interchange
F_3	1	1	6	2	7	3
F_4	3	4	0	1	3	5
F_5	0	7	1	1	1	8
F_6	1	0	7	0	8	0
F_7	0	5	4	7	4	12
Total	5	17	18	11	23	28

cally and the two homozygotes according to whether they are yellow or green.

As mentioned earlier, yellow phenotypes are absent from line *2*. At meiosis in the F_1 or F_2 there must consequently have been an exceptional recombination between *y* and the point of interchange, presumably at a proximal chiasma. The decrease in chiasma frequency with further inbreeding (see Rees, 1955) no doubt explains why a similar recombination has not eliminated the *y* gene from lines *1* and *3* during the course of the experiment.

It was also suggested earlier that an exceptional proximal chiasma, such as is postulated to have occurred in line *2* would decrease the genic heterozygosity in structural heterozygotes. It is therefore possible that the smaller advantage of structural heterozygotes in this line may partly be a result of their reduced heterozygosity.

Old and new homozygotes. The frequencies of green-leaved plants homozygous for the interchange chromosome *A* are distinguished from the heterozygotes by cytological examination. Because their estimation is limited to samples classified cytologically, comparable estimates of the yellow plants, homozygous for the normal chromosome *A*, are also based on the same samples of plants subjected to cytological examination. The frequencies are given in table 3.

From the grand totals in the table it can be seen that the numbers of the two kinds of homozygotes are approximately equal. Clearly the excess of the *A* heterozygotes demonstrated in the previous section cannot be attributed to a lethal or sub-lethal homozygote. On the contrary, its superiority over both homozygous classes confirms an advantage based on heterozygous genetic balance, probably of a polygenic nature.

The table shows also that the ratios of the two homozygous classes are different in the two lines. The heterogeneity x^2 is 7.75 and significant at the 1 per cent level. There is therefore some indication that the survival of the old and new type of chromosome in the homozygous condition is influenced by the background genotype. Whether this interaction with background relates directly to the consequences of structural change, e.g., a position effect, or to genes such as the *y* gene associated with the changed chromosomes, cannot be established.

5. DISCUSSION

When structurally new types of chromosomes arise by interchange or inversion in natural populations, their maintenance largely depends on their effects upon two, sometimes conflicting, properties; first, the *survival* of individuals and, second, their *fertility*—the latter almost invariably being reduced in structural heterozygotes. In outbreeding populations where structural heterozygotes will be inevitable and also in inbreeding populations if structural heterozygosity is otherwise advantageous, the infertility may be a very considerable limitation.

In interchange heterozygotes the cause of fertility is genetic non-disjunction at first anaphase of meiosis. This in turn depends on the type of orientation of the interchange rings and chains at first metaphase—one type leads to non-disjunction and sterility, the other to disjunction and fertility. For

the interchanges described in this paper Thompson (1956) has previously shown that the proportions of disjunctional as opposed to non-disjunctional arrangements of the chromosomes are genotypically controlled, and, as would then be expected, can be altered by selection for higher fertility (Lawrence, 1958). We may therefore conclude that the genetic system of a population can to some degree at least become adapted to accommodate such structural changes without disastrous loss of fertility.

As to the consequences of change upon survival, the present results must of course be considered in relation to the breeding system imposed during the experiment. The rye lines were forcibly inbred and selection, not unexpectedly under these circumstances, favored the heterozygous gene combinations preserved by and associated with structural heterozygosity. Bearing in mind the nature of the breeding system, two main factors were shown to influence the genetic consequences and hence the distributions of the interchanges studied.

In the first place, while heterozygotes of one interchange, *A*, were favored by selection, those of *B*, were not. These results confirm those briefly reported for early inbred generations reported by Thompson and Rees (1956).

Secondly, the consequences of selection vary according to the background genotypes with which the structural types occur. It was shown that the advantage of *A* heterozygotes increased with increasing homozygosity of the other chromosomes. Also, selection for chromosome types varied between lines—reflecting the influence of different genotypes on the expression of similar gene combinations.

When we turn to the records on interchanges in wild populations, we find that in two groups, *Oenothera* (Cleland, 1949), and in cockroaches (Lewis and John, 1957) the high incidence of interchange heterozygosity is associated with inbreeding. In *Oenothera* Californian populations are outbreeding and characteristically free of interchange heterozygotes. In populations to the east and north inbreeding, imposed no doubt originally by isolation during colonization, becomes the rule and, with it, extreme structural heterozygosity (cf. Darlington, 1956). In cockroaches also it is in isolated inbred populations that interchange heterozygotes become common. The experimental results with rye, like those previously described by Darlington and La Cour (1950) in *Campanula*, show how structural heterozygotes, by preserving genic heterozygosity, are at an advantage with enforced inbreeding.

Finally, in natural populations we find different structural combinations to be localized (e.g., Cleland, 1949) and presumably adaptive in those localities. Experimentally, an exactly parallel situation is revealed by the different adaptive properties of the interchanges investigated.

6. Summary

1. In rye the heterozygotes of one of two new interchanges had a selective advantage over homozygotes. The advantage was attributed to genetic balance of genes in a heterozygous combination preserved by the structural change.

2. The relative advantage of heterozygotes increases in proportion to the degree of homozygosis of chromosomes not involved in interchange.

3. The survival properties of both heterozygotes and homozygotes vary in inbred lines of different genotypes.

7. Acknowledgments

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CONDITIONS FOR THE EXISTENCE OF BACTERIOPHAGE[1]

ALLAN CAMPBELL
Department of Biology, University of Rochester

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Bacteriophages (phages) are viruses whose hosts are bacteria. The ecology of bacteria and their phages has been studied less thoroughly than that of many higher organisms. The experimental data on their behavior in the laboratory are as extensive as for almost any other living things. Experimentation has been oriented primarily at answering basic biological questions, with phage serving as a convenient tool for technical reasons. Several facts have emerged from it, however, which are important to an understanding of the position of phages in the biological world and their evolution and survival in nature.

We shall first describe some general properties of phages, and then list some specific facts which are now sufficiently well established to serve as a basis for critical discussion. We shall then attempt an analysis and evaluation of the possible factors which may be responsible for the perpetuation of phage.

It is convenient to start by classifying phages into two types: *Virulent* phages can reproduce only by destroying their host. *Temperate* phages are in addition capable of multiplying indefinitely as intracellular parasites within the same cell lineage. The distinction can be made only with respect to a given host species; a phage may be temperate on one host and virulent on another (Bertani, 1958). Also, virulent phages can arise as mutants of temperate phages.

When a virulent phage infects a bacterium susceptible to it, the cell dies, produces more phage, and lyses. When a temperate phage infects a sensitive cell, the result is sometimes the same as with a virulent phage. However, in a fraction of the infected cells, a different pathway is followed. The cell does not die or lyse. Far from being unaffected by the encounter, however, these cells undergo a permanent, hereditary change. Each of their descendants can give rise to a clone of cells which are specifically immune to lysis by external infection with the type of phage which had previously entered their ancestor; and, in any such clone, a small constant proportion of cells lyses each generation and liberates into the medium new phage of the original type. Such strains are called *lysogenic*, and the noninfective, intracellular form in which the capacity to liberate a particular phage is reproduced is called *prophage* (Lwoff, 1953).

IMPORTANT FACTS

1. *Bacteriophages are plentiful in nature.* Modern bacteriophage research has been concentrated on a few phages which attack various strains of the Enterobacteriaceae. Such intensive study has been designed to achieve depth rather than breadth. However, even a cursory examination of the literature reveals that, for almost all the major groups of bacteria, there are corresponding phages able to grow on them. From almost any natural environment such as sewage, soil, etc., where bacteria are common, phage are common also. For the Enterobacteriaceae a number of distinct species of phage are known to exist, each of which appears to be reproductively isolated from the rest.

Perhaps more impressive than the widespread occurrence of phage itself is that of lysogeny. A full appreciation of how extensively phages have established themselves as prophages comes more from experimental laboratory studies than from screening of direct isolates from nature, although the former procedure yields results

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which are already suggestive. This is because lysogeny is not an overt property of a bacterial culture. The cells themselves are immune to the phage they liberate. Since the amount liberated is in general too small to find by visual observation (i.e., electron microscopy), one observes that a strain of bacteria is lysogenic only when one is in possession of a related strain which is not lysogenic for the phage in question but on which it is able to grow.

Sometimes, such related strains have been obtained only as derivatives of the original lysogenic culture which have been cured of the prophage. Such cured individuals very rarely arise spontaneously, but their frequency is higher among the survivors of heavy doses of ultraviolet irradiation.

The observation of Zinder (1958) illustrates the point well. Zinder was studying the growth of a phage (which can be called phage PA) on strain A of *Salmonella typhimurium*. When phage PA was grown on strain B of *S. typhimurium*, one obtained a relatively high proportion of phage which were variants for various morphological characters. Apparent multiple mutants occurred, suggesting that their origin was really recombinational rather than mutational. The observation was explainable if one assumed that strain B was really lysogenic for a prophage which was related to phage PA. Zinder therefore irradiated strain B, and selected among the survivors for colonies showing signs of being lysed. When isolated in pure culture, the bacteria from these colonies proved indeed to have lost a prophage; i.e., the phage PB could be demonstrated in filtrates from strain B by plating on its cured derivative B1.

Phage B would not grow on strain A. However, strain A itself proved to be lysogenic for another phage (PB1), and when strain A was cured of PB1, a new strain SA1 was obtained on which phage PB would grow. Strain B was also found to be lysogenic for two other prophages (PB2 and PB3).

One could cite many other examples where a given bacterial strain has been discovered to carry one or more prophages only after the strain has been approached with the proper tool (in this case, superinfection by the related phage PA). The results of Anderson (in Adams, 1959) indicate that most of the *Salmonella* types encountered in epidemiological work are lysogenic for one or more prophage. It must also be mentioned that prophage can become even more deeply hidden within the bacterial genome than in the normal type of lysogeny. *Defective lysogenic* strains may be considered in this connection.

As mentioned above, lysogeny has always at least two attributes: (1) the ability to liberate phage and (2) the immunity to superinfection by the carried phage type. Many instances are known in which, from such a truly lysogenic strain, there have been isolated mutant substrains which have lost the first property but retained the second. Careful genetic study has revealed that, in general, each such defective lysogenic strain carries a defective prophage, which has mutated from the wild type at some one genetic locus, and which, although still able to reproduce as prophage, is unable to carry out all the steps in the normal lytic cycle (Jacob et al., 1957).

If careful scrutiny is required to detect lysogeny, the same is doubly true for defective lysogeny. In general, defective lysogeny has been demonstrated only when the strain in question has happened to be infected with a phage related to the prophage it carries, and even then it may frequently be overlooked.

A good example comes from a study by Bertani and associates (Bertani, 1957; Cohen, 1959) of the growth of phage P2 on strain B of *Escherichia coli*. When phage P2 is grown on this strain and then plated on another bacterium (*Shigella*), a small percentage of plaques are recognizably different from the majority by virtue of their small plaque size. The phage

from these plaques are immunologically similar to phage P2 and can undergo genetic recombination with it, but can be distinguished from it in several ways. The most interesting property is that, whereas bacteria lysogenic for P2 prophage are immune to superinfection by phage P2, the new phage (which has been named P2 *dismune*, or P2 *dis*) forms plaques on strains lysogenic for P2. Virulent mutants of phage P2 are known, but P2 *dis* is not virulent. It can lysogenize *Shigella*, and strains of *Shigella* carrying P2 *dis* as prophage are immune to superinfection by phage P2 *dis* but not by phage P2.

Strain B does not liberate any P2 *dis* except when superinfected by phage P2. It is, however, immune to phage P2 *dis*. If strain B is infected with phage P2, some survivors can be isolated which have been "cured" of their immunity to P2 *dis*. All these facts are most readily explained on the assumption that strain B is lysogenic for a defective prophage related to P2 but different from it at several genetic loci, including the one(s) controlling the specificity of the immunity.

The system raises a conceptual danger which we will elaborate later—namely, that of assuming the presence of a whole corresponding to an observed part. The "defective prophage" P2 *dis* is postulated by virtue of its ability to yield a new phage type by recombination with an active phage P2. The term may well be as misleading as if we were to refer to one molecule of pneumococcal DNA as a "defective bacterium." Certainly a small piece of the genetic material of *E. coli* B can become part of a phage, but as we shall see later, this property does not necessarily distinguish it from any other part of the bacterial genome.

2. *Bacteriophages are structurally complex*, apparently more so than animal or plant viruses. The T-even phages have been studied most thoroughly. The DNA, together with a substantial amount of various polyamines, surrounded by a protein coat, form a polyhedral "head," to which is attached the "tail." The latter represents an intricate piece of biological engineering (Brenner, et al., 1960). Surrounded by a contractile sheath layer is a hollow "inner core," at the end of which are numerous fibers. Infection apparently is initiated by the attachment of these fibers to specific receptor sites on the surface of the bacterium. Digestion of thiolester bonds in the phage tail by an enzyme of the bacterium is followed (in some order) by release of a lysozyme-like enzyme contained within the phage tail which digests a portion of the bacterial cell wall, contraction of the sheath, and injection of the DNA from the head, through the center of the tail, into the bacterium (Garen and Kozloff, 1959).

Once the DNA has entered, the whole protein structure is superfluous to the further development of the phage. The bulk of the protein actually remains outside the infected cell. Mixing the suspension in a Waring blender at this point shears off the protein of the infecting particles without impairing the ability of the cells to reproduce more phage (Hershey and Chase, 1952).

Each of the various protein fractions (from the head, the contractile sheath, and the tail, respectively) has been purified to some extent. Each individual structure seems to be composed of an array of identical molecules. For example, the sheath is composed of approximately 200 molecules of molecular weight 50,000. The protein of the head is, however, different antigenically and physically from that of the tail fibers, which is in turn different from that of the sheath. The structural specificity of the fiber protein is determined by the phage genome, and this is probably true for the other proteins as well.

3. The genome of a temperate bacteriophage can reproduce under at least two conditions. It can multiply rapidly and autonomously, in which case causes a variety of events to occur, resulting in the synthesis and assembly of all the parts of the mature phage, and also in the lysis of

the cell. Alternatively, it can multiply as a prophage, in which case it is integrated into the bacterial genome, and multiplies at the same rate as the cell. Until recently, phage were the only things with both these potentialities.

Now it has been shown that some genetic elements other than phage can also multiply in an autonomous or an integrated state. Such elements have been named "episomes" (Jacob and Wollman, 1958a). The episomes other than phage genomes so far discovered are the fertility (F) factor of *Escherichia coli*, and the factor responsible for producing colicine K30. The F factor illustrates the point.

Wild type *Escherichia coli* K-12 contains the F factor, and is called F^+. Certain substrains have lost it, and are called F^-. Operationally, the two states are distinguished by the facts that two F^- strains produce no recombinants when mixed, and that $F^+ \times F^-$ crosses are more fertile than $F^+ \times F^+$.

The F factor is infectious, but only by direct cellular contact. When a few F^+ cells are mixed with an F^- culture, the rate at which F spreads through the population is such that one must assume it can multiply more rapidly than the bacteria carrying it. This proves it can, under some circumstances, multiply autonomously.

From F^+ strains can be isolated rare substrains (Hfr). Hfr $\times$ F^- crosses are tremendously more fertile than $F^+ \times F^-$. When a culture of Hfr cells is mixed with an excess of F^- cells, each Hfr cell forms a conjugating pair with an F^- cell, and the genetic material of the Hfr cell enters the F^- cell in an oriented manner, the F factor entering last. Each independently isolated Hfr is unique in the particular genes which are transferred first (Jacob and Wollman, 1958b).

The formation of Hfr strains is therefore most simply interpreted as the integration of F at a particular point of the bacterial genome. It can be integrated at any one of a number of different points, perhaps at any point.

One could, of course, argue that F is really a phage or a defective phage. At the moment, such usage can only cause semantic confusion. The F factor is not transmissible by culture filtrates of F^+ cells,[2] and vegetative phage are not transmissible during cellular conjugation (Jacob et al., 1960).

4. Some phages, even in the prophage state, cause the cell to carry out specific syntheses not obviously connected with the synthesis of phage. The term *lysogenic conversion* has been given to the acquisition of new metabolic properties by a bacterium when it is lysogenized by such a phage. For example, cells of *Salmonella typhimurium* do not normally make a particular protein (antigen 15). However, cells of this species infected with a phage (ϵ^{15}), or cells carrying this phage as prophage, do manufacture this protein (Uetake et al., 1958).

Some temperate phages can mediate the transfer of bacterial genes from one host to another. If the phage P22 is grown on a strain of *S. typhimurium* which is, for example, streptomycin-resistant and histidine-independent, the lysate thus prepared can transform a small proportion of the cells of a streptomycin-sensitive, histidine requiring strain to streptomycin resistance, and another small proportion to histidine-independence. These transformations are permanent. The characters *transduced* by the phage have become hereditary properties of the transformed strains, which behave in every way as though the genetic determinants for these characters had replaced their homologues in the bacterial recipient (Zinder, 1955).

At first, this transduction of bacterial genes seemed fundamentally different from lysogenic conversion because (1) the transformed recipient need not have become lysogenic in the process (2) any bacterial gene can be transduced and, (3) the transducing activity for a given gene is completely abolished by one cycle of

[2] The recent finding of Arber that F can be transduced by phage P1 constitutes a not completely trivial exception to this statement.

growth on a host not containing that gene. The discovery that *phages and other episomes can incorporate bacterial genes by some process of genetic recombination* makes sharp demarcation between the two impossible.

The first demonstrated case was the transduction of the galactose genes of *Escherichia coli* K12 by the bacteriophage λ (Morse et al., 1956). Unlike phage P22, λ does not transduce any genetic markers from the host of the previous growth cycle. However, if a phage lysate is obtained from a strain lysogenic for λ, it will transduce some genes controlling galactose fermentation which are located on the bacterial chromosome close to the specific site at which the λ prophage is integrated. The transducing activity has proven to be associated not with the active phage particles themselves, but with a new type of particle which appears in the lysates which is genetically part phage and part bacterium.

The new particles resemble the λ phage in their serological properties and their specificity of adsorption to particular bacterial strains. They therefore presumably have the same type of protein coat and tail. Genetically, however, they are missing a section of the phage genome comprising about 30% of the known genetic map, which is taken out from the middle of this map. Instead of this, they contain the genes for galactose fermentation which originally behaved as purely bacterial genes.

These new particles, which we can call λ-gal, are thus very similar to converting phages. The only difference is that they are defective and therefore can be reproduced only as prophage unless an active λ phage is introduced into the same cell.

In transduction of other genes by other phages, such defective transducing phage have likewise been implicated (Luria et al., 1958).

This ability to incorporate genes of the host by recombination is not restricted to phage. The fertility factor can also incorporate various bacterial genes, which subsequently behave as though part of the F factor itself (Jacob and Adelberg, 1959). Whereas there is as yet no case in which a phage becomes a (non-defective) converting phage by recombination with the bacterial chromosome, there seems to be no reason to think that this cannot happen.

Population Dynamics of Phage

The Problem.—We wish to ask how phages perpetuate themselves under natural conditions. To be conservative, we will first inquire whether the question is a real one.

The fact that a free-living organism occurs in nature at all constitutes *prima facie* evidence that it is a representative of a species which can maintain itself in some ecological niche. For viruses, which have, as we have already seen, a certain tendency to become scrambled up with the non-viral part of the host genome, one might assume that the viruses we find are always transient offshoots of something not itself a virus, and that the virus itself is not perpetuated as such over long periods of time.

Two facts, in our opinion, do suggest a long evolutionary history of phages *as phages*. The first is their abundance. The second is their complexity. It is hard to imagine the development of the highly specialized system of organelles peculiar to phage unless a structure adapted to perform specifically viral functions had been selected for over a long period of time. We will therefore assume that, like other organisms, phage must be able to establish some conditions reasonably approximating a steady state in which the size and composition of the population remain unchanged from year to year or from millenium to millenium. There are several possible ways in which this might happen.

(1) *The simple predator.* If a virulent phage and a susceptible bacterium are mixed in an open growth system, such as a chemostat (Novick, 1955), one expects that, in general, the concentration of the two will approach some stable steady state

value. By making some oversimplified assumptions, we can discuss this quantitatively.

Let P be the concentration of phage; B, the concentration of bacteria; a, the flow rate constant; k_A, the adsorption rate; and K_I, the rate of spontaneous inactivation of phage. Let the normal growth law of the bacteria be logistic with growth rate k_B and maximal level L. The bacterial concentration will then change with time according to

$$\frac{dB}{dt} = k_B B\left(1 - \frac{B}{L}\right) - aB - k_A PB \quad (1)$$

The equation for phage concentration is slightly more complex. If each infected cell yields N phage particles at a time l seconds after infection, then

$$\frac{dP}{dt} = k_A N\left[B(t-l)P(t-l)\right] - k_A BP - k_I P - aP \quad (2)$$

where $B(t-l)$ and $P(t-l)$ are the values of B and P at time $t-l$.

At the steady state, $\frac{dB}{dt} = \frac{dP}{dt} = 0$ and also $[B(t-l)][P(t-l)] = BP$.
So

$$B[k_B\left(1 - \frac{B}{L}\right) - a - k_A P]$$
$$= P[k_A(N-1)B - k_I - a] = 0 \quad (3)$$

There are four possible solutions.

(i) $B = 0$, $P = 0$

(ii) $B = 0$, $k_I + a = 0$, $P = P_0$

(iii) $P = 0$, $B = L\left(1 - \frac{a}{k_B}\right)$

(iv) $B = \frac{k_I + a}{k_A(N-1)}$

$$P = \frac{k_B}{Lk_A}\left[L\left(1 - \frac{a}{k_B}\right) - \frac{k_I + a}{k_A(N-1)}\right]$$

Whenever the initial populations are not zero and either the flow rate or the rate of spontaneous inactivation of the phage is greater than zero, either solution (iii) or (iv) will be chosen, depending on whether the steady state phage concentration of (iv) is positive or negative; in other words, if the phage grows too slowly, it will die out, but if it grows rapidly, it will maintain the bacteria at a low, non-zero level.

Since we are considering survival in nature, it is reasonable to suppose that the host competes with other species occupying the same ecological niche, and we can ask whether the phage will in this case decrease the selective advantage of its host relative to its competitors.

Denote the host by B_1, and the competing species by B_2. Let $B = B_1 + B_2$. The equations become

$$\frac{dB_1}{dt} = B_1[k_{B_1}\left(1 - \frac{B}{L}\right) - a - k_A P] \quad (4)$$

$$\frac{dB_2}{dt} = B_2[k_{B_2}\left(1 - \frac{B}{L}\right) - a] \quad (5)$$

$$\frac{dP}{dt} = k_A N[B_1(t-l)P_1(t-l)] - k_A PB_1 - k_I P - aP \quad (6)$$

Proceeding as before, we find that, if $k_{B_1} \leq k_{B_2}$, then always $P = 0$ at the steady state. However, if $k_{B_1} > k_{B_2}$ a steady state is possible at

$$B_1 = \frac{k_I + a}{k_A(N-1)} \quad (7)$$

$$B_2 = L\left(1 - \frac{a}{k_{B_2}}\right) - \frac{k_I + a}{k_A(N-1)} \quad (8)$$

$$P = \frac{a}{k_A}\left(\frac{k_{B_1}}{k_{B_2}} - 1\right) \quad (9)$$

Now, in the absence of phage, the faster-growing bacterial species will always displace the slower. The only case in which the net effect of the presence of phage is to create a selective disadvantage for its host is when the two growth rates are exactly equal. When the host for the phage has a selective advantage, even a very slight one, the competitor has no effect on the final density of the host bacterium or

on the stability of the steady state. It merely fills up the space which the susceptible species leaves vacant, and, indirectly, reduces the level of phage.

Reasoning in this way, one can conclude that a phage can survive without destroying its host simply by having chosen the best-adapted from a group of competing hosts. We shall discuss the limitations of this conclusion later.

(2) *Continual variation.* The simplicity of the phage-host system, together with the ease of making measurements, suggest an ideal situation for studying predator-prey equilibria. In fact, things are not so simple because bacteria can mutate to phage-resistant types, most commonly to types whose surface properties have changed so that the phage is no longer able to attach. Before an equilibrium can be established, therefore, the culture becomes overgrown with such mutants.

This does not necessarily lead to the elimination of phage from the system, because the phage can mutate to a form able to attach to the resistant bacterium. The process can continue by the occurrence of a new bacterial mutant resistant to the new phage, and another phage mutant capable of growth on the latest bacterial strain. One can imagine an indefinite series of such fresh invasions of previously resistant hosts, followed by the emergence of correspondingly new bacterial types. Such evolution could give rise to an infinite series of new types, or, as long as one considers finite populations[3] of the sizes likely to be encountered either in nature or in the laboratory, it could equally well be circular, in that the n^{th} bacterium would be again susceptible to the original phage, which then could emerge as the $(n+1)^{st}$ phage in the series (cf. Atwood et al., 1951).

Inasmuch as such mutations occur in the laboratory, they undoubtedly occur also in nature, and almost certainly any natural population of virulent phage at any given time and place owes its immediate survival to the latest in such a series of steps. It is much less clear whether the long-range survival of phage depends purely on such a sequence of hops, skips, and jumps.

The resistant mutants may have a slight selective disadvantage compared to the parent strain. This point has not been carefully studied, but, since most mutations of a highly evolved organism are anti-adaptive, this is a real possibility. As shown above, introduction of the resistant mutant would not, in this case, destroy the original predator-prey balance.

(3) *The effect of periodic selection.* The conclusion that phage does not confer a selective disadvantage on a host which has initially a selective advantage has been mathematically demonstrated above. It is only valid, however, so long as the conditions of the derivation are fulfilled. In particular, these are violated as soon as one introduces the possibility that both the host and the competing species can undergo adaptive mutations unrelated to phage growth. In this situation, the probability that such a mutation will happen within a growing population during a given time interval is proportional to the number of individuals in that population.

The more adapted mutant individual and its progeny will then gradually begin to overgrow the whole culture. In an infinite population, each "more adapted" mutant of the competing species could set up a new equilibrium with its counterpart among the host for the phage. However, in a finite population there is always the possibility that the phage-sensitive species will become extinct before this happens. Since the competing species with an initial selective disadvantage could never have developed at all in the absence of phage, the phage obviously will tend ultimately to eliminate its host, and therefore, itself.

The selective overgrowth of a bacterial culture by a better-adapted mutant arising during the course of the experiment has frequently been observed in continuous

[3] In a population of infinite size, the oscillations characterizing circular evolution become damped and a single steady state is ultimately approached.

culture studies (Novick, 1955; Atwood et al., 1951). Generally, it has made impossible the experimental determination of mutational equilibria and would be expected to interfere likewise with the measurement of predator-prey equilibria among microorganisms.

(4) *The scavenger hypothesis.* Up to this point, we have assumed that the host is faced with the problem of survival in the presence of the phage; i.e., that bacterial cells killed by the phage are those whose multiplication would otherwise lead to the perpetuation of the species. In point of fact, we know too little about bacterial populations in nature to make such an assumption.

By destroying a portion of the host population, a parasite will decrease the probability of long-range survival of that population, provided that the individuals destroyed by the parasite would otherwise have contributed to the perpetuation of the species. If the parasite affects individuals which for some reason are already destined to die without issue, the net effect may be instead indifferent or even beneficial.

Although phage will, under some circumstances, grow on non-viable cells (for example, those killed by ultraviolet), the chemical conditions which permit good growth of phage are in general the same as those which favor rapid proliferation of the bacteria on which they are growing. This makes highly unlikely any selective growth of phage on dead or dying cells within a culture.

The natural situation is less clear, because we do not really know, in general, how the bacterial population perpetuates itself. It is quite possible that phage growth might occur on cells which are "effectively dead" from the genetic point of view and spatially isolated from the "germ line" of the population.

Consider, for example, the following purely hypothetical case: A bacterium lives and reproduces in the intestine of some animal. It is transferred from animal to animal by direct contact. It is voided in the feces and thus enters soil, water, or other habitats in which it can grow extensively but ultimately perishes as a result of competition with other species indigenous to these habitats. The destruction of these bacteria by phage in any habitat other than the intestine obviously cannot decrease their survival as a species; any more than a neurotropic animal virus which affects exclusively animals past the reproductive age can impart any selective disadvantage to its host.

The benign parasite. Temperate bacteriophage.—As mentioned at the outset, many species of phage are capable not only of multiplying at the expense of the host cell, but also of becoming prophages, which perpetuate themselves as part of the host cell genome.

The term prophage was originally used (Lwoff, 1953) to denote the non-infectious form in which the phage is reproduced in lysogenic strains. It was subsequently found that some prophages, such as λ and P2, are assorted during bacterial recombination exactly like genes of the host cell (Lederberg and Lederberg, 1953; Bertani and Six, 1958). The term is now generally used to refer to this state of the phage genome in which it is integrated into the bacterial chromosome.

It is not yet clear whether the integrated state is the only one in which a phage genome can multiply indefinitely within a bacterium. There are some prophages for which no genetic localization has been possible, and it has also been shown that, at least for a number of generations, an infected cell can multiply in a "carrier" state, in which the phage genome has not yet stably established itself (Zinder, 1958).

For our purposes, the main interest of lysogeny is that it provides a means by which a phage can perpetuate itself without harming its host in such a violent way as a virulent phage. What we must inquire is under what circumstances we can really expect this apparently innocuous behavior to aid in the phage's survival.

Suppose that the presence of a prophage

does not affect in any way the metabolism of the host, except insofar as material and energy are needed to synthesize the prophage and any substance necessary for its maintenance. The presence of the prophage would then be expected to cause a small decrease in the reproductive rate of the host, since it must now divert a portion of its synthetic energies to making a certain amount of dead weight. Good data on this point are lacking, although a large effect on the growth rate of the cell by the prophage seems excluded in most cases.

At any rate, it is clear that a metabolically inert prophage would, in general, impart a selective disadvantage to its host, which should lead to the eventual elimination of both through competition with hosts which do not carry prophages. One therefore must look for possible means by which the phage might impart a selective advantage to its host.

The advantage might occur either at the cellular level or at the populational level. In the former category, all we can say is that some properties acquired by lysogenic conversion *could* impart a selective advantage under the proper conditions. In the latter category we can place transduction. It is easy to see how transduction can increase the selective value of the host species by permitting the formation of recombinant types adapted to a particular local environment. Furthermore, since transduction will most commonly occur where much phage is present, the selected recombinants have a good probability of being lysogenized and thus perpetuating the phage. It thus appears easier to explain the existence of temperate phages than of virulent ones, and it has been suggested that all phages are really temperate, provided one finds the proper host.

One problem is that temperate phages can give rise to virulent mutants, some of which can destroy even the lysogenic cells. Under conditions in which phage can grow well, these virulent mutants should set up the same sort of equilibrium with the lysogenic cells as a virulent phage would with sensitive cells. Those cells which did survive would have, however, the advantages of recombination imparted to them by the temperate phage.

It is possible that the natural growth conditions of the host are such that the virulent mutants will die out rather than spreading through the population. This is perhaps an overly facile solution. The virulent mutants would fail to be self-sustaining under conditions in which the burst size is small, or the adsorption rate is low, or the rate of spontaneous inactivation is high. Any one of these would also reduce the efficacy of the temperate relative as a vector in recombination. The most adaptive situation will be one in which an infinitesimal change in the growth properties of the phage would have two effects equal in magnitude but opposite in sign, the one to increase the adaptive value of the host by producing more recombinants, the second to decrease it by reducing the total number of individuals and increasing the number of competitors.

If the balance does permit the virulent mutant to be self-sustaining, we must then note that the critical relationship between the virulent mutant and its temperate relative is not their common genetic origin but rather the similarities in their growth properties. A virulent phage completely unrelated to a given temperate phage could set up the same kind of balanced relationship with it provided that the specificities of adsorption and the other requirements for growth were identical.

The critical reader may feel that this possibility simply underscores a certain carelessness in our whole approach up to this point, in the tacit assumption that sensitivity to a given phage (e.g., the virulent phage of the above paragraph) is a property capable of mutating independently of any other property (e.g., sensitivity to the temperate phage of the above paragraph), which might be of value to the host. We can offer no real defense against this charge. The type of mimicry described above does seem to us to create a situation

unusually demanding on the bacterium's powers of discrimination, however.

We must avoid leaving the impression that transduction by virulent phages is impossible. Indeed, Starlinger (1958) has shown that a virulent mutant of phage P 22 can mediate transductions into sensitive recipients, provided that the multiplicity is very low and later superinfection is avoided. In this system, the particles which transduce seem not to contain a full phage genome and may lack any phage genetic material at all.

In order that the ability to transduce should help sustain a virulent phage in nature, the advantage conferred on the host population by the one particle in, say, 10^3, which transduces, must outweigh the killing of 999 cells by the phage itself. Our discussion of the simple predator-competitor system suggests that this is quite possible. If the host and the competitor initially had equal adaptive value, and if that of the host is increased more by recombination than that of the competitor by mutation, the phage will then help to perpetuate its host. Temperate phages are better suited as vectors in transduction only under circumstances where the cell is likely to be superinfected at or near the time of transduction by an active phage particle; i.e., under conditions of high multiplicity.

A phage genome is a special type of episome. Episomes in the integrated state behave as bacterial genes. It is an old idea that perhaps every bacterial gene has a certain potentiality to become a phage (Lwoff, 1953). If this were so, natural selection among temperate phages could almost be reduced to the selection of particular genes within the bacterial population.

Now, the fact that phages can incorporate bacterial genes is operationally equivalent to saying that each bacterial gene can become a phage. If λ-gal had been discovered before bacteriophage λ rather than afterwards, it might easily have been mistaken for a phage-like form of the *gal* gene. It is easy to imagine situations where one would have no means whatever of distinguishing the process of "becoming a phage" from that of "becoming part of a phage." We can thus expect that, as a practical matter, every bacterial gene has the potentiality to exist not only in a transmissible cell-free form (as was shown already in studies of transformation and common transduction) but also to replicate in either an autonomous or an integrated state.

How can we reconcile this statement with the idea that phages have evolved their structural complexity *as phages*? Each phage contains a certain number of genes which perform functions specifically phage-like, such as the lysis of the cell, the production of phage coat protein, the breaking down of bacterial nucleic acid, etc. These genes work together to produce as an integrated effect the production and liberation of new phage particles, and it is hard for us to imagine that a fortuitous combination of genes previously dedicated to other tasks (or even integrated into different patterns) could often become this well integrated. It is only to these genes, not to the phage genome as a whole, that we must ascribe a long history of associated existence in which the phage functions were selected for.

We also may assume that the average phage contains some genes which do not perform specifically phage-like functions and which have been selected for their ability to perform functions in the bacterial cell which increase the adaptive value of the bacterium. Each species of phage can then be thought of as such a symbiotic association of "phage genes" and "bacterial genes." As in any problem of evolution, rigid qualitative distinctions can be expected to break down beyond a certain point. Rare deviants can extend the limits of the species, arising not only by mutation in the conventional sense, but also by incorporating small pieces from the great reservoir of host genetic material always available. In some cases, a bacterial gene accidentally acquired may serve a useful function in the lytic cycle of the phage; sometimes a "phage" gene may, as pro-

phage, produce something of value to its host. We would, however, expect that, given adequate information, one would find no conceptual bar to classifying the large majority of the genes of a given phage as either "phage" or "bacterial" depending on the type of function for which they have been selected.

Under given local conditions, short term selection may favor forms in which the viral function has been exaggerated to give a completely virulent phage; conversely, conditions which select only for favorable bacterial genes acquired through transduction or conversion will lead to the gradual accumulation of defective prophages within populations originally truly lysogenic. The long range survival of the species depends, however, on the presence in the phage population of individuals which combine both functions.

The hypothesis has been proposed (Adams, 1959) that phages represent degenerate forms of bacterial recombination systems. This amounts to about what we have said here, except that there seems no special reason for thinking them degenerate. They are, rather, less completely integrated into the genetic apparatus of the host cell than are the factors which determine fertility in higher organisms; and their evolution can therefore be considered separately from, although not independently of, that of their hosts.

The function of specialized structures in phage.—Two items merit more critical discussion than we have given them. The first is the function of the specialized structure of phage. We will assume that our present interpretation of what these structures do is correct, but will inquire how they may help the phage to survive.

The three major components are the DNA, the head protein, and the tail protein. The presence of DNA requires no justification. The head protein protects the DNA from deleterious agents in the environment.

The tail causes the transfer of DNA from within the phage head into the bacterial cell. It thus might serve two purposes (1) to introduce the DNA into the bacterium and (2) to allow the DNA to remain protected within the head protein until such time as it is in the vicinity of a recipient bacterium. In the second case, the tail would serve primarily as a sensory organ which informs the DNA of the most propitious moment to emerge from its "shell"; and the fact that the DNA may be injected *into* rather than *adjacent to* the bacterial cell might be a purely accidental concomitant of the ejection process.

The experimental evidence is not decisive. It would seem important for a phage to have a special penetration mechanism only if the entrance of the free DNA would be a difficult process. There are no cogent reasons for supposing that it would be. Certainly, DNA must be able to enter bacteria of those species for which transformation has been demonstrated. In at least some cases where transformation is not readily shown, the difficulty lies not in the penetration of the recipient but rather in the degradation of DNA by nucleases from the donor (Schaeffer and Ionesco, 1959).

Reproductive isolation and speciation in phages.—The idea that the consequences of reproductive isolation are the same among bacteria as among higher organisms has recently been discussed in detail (Ravin, 1960). The same considerations apply to phage.

For phage, reproductive isolation can occur at at least three different levels. (1) The two phages may not have a common host. (2) When a suspension of cells of the common host is mixedly infected with the two phages, each cell liberates on lysis only one or the other type, never both. This is called *exclusion*. (3) In at least one reported case (quoted by Adams, 1959), two very dissimilar phages will multiply in the same cell at the same time without undergoing genetic recombination.

Generally, exclusion is exhibited between phages which differ from each other morphologically and immunologically. Those

phages which multiply in the same host at the same time are generally similar in these respects, and will recombine with each other. The mechanism of exclusion is not known. Whether it is a cause or a consequence of genetic isolation is also unknown. It does not of itself preclude recombination, since the excluded phage does attach to and probably penetrates the recipient cell.

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Summary

A. The implications of experimental findings with phages on our ideas about their evolution are discussed. Four facts are emphasized as of especial importance.
   (1) Phages are plentiful in nature.
   (2) They are structurally complex.
   (3) Besides temperate phages, there exist in bacteria other elements capable of multiplying in either an autonomous or an integrated state (episomes).
   (4) Phages and other episomes can incorporate bacterial genes.

B. Various possible explanations for the continued existence of bacteriophage are examined. It is concluded that:
   (1) Phages probably have evolved *as phages* rather than representing transient offshoots of some other evolutionary line;
   (2) Their role in bacterial recombination may be the most important factor in assuring their survival.

C. The function of some of the specialized structures of phages is discussed briefly, as is the question of reproductive isolation and speciation.

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ECOTYPIC DIFFERENTIATION: OBSERVATIONS AND REFLECTIONS

J. W. Gregor and Patricia J. Watson
Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, Scotland

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The ecotype concept (Turesson, 1922) is a population concept, and by definition an ecotype is an intraspecific product of environmental selection arising as a result of genotypic response to a particular habitat. Accordingly, before a population qualifies for ecotype status, a relationship must be demonstrated between its heredity and the prevailing factors of its habitat. Since the primary object of the early genecological studies was to demonstrate the existence of ecotypic differences, it was only natural that the comparisons made then were usually between population samples collected from distinctly different habitats. The results of such experiments showed the validity of the ecotype concept, though as subsequent investigations bear witness they tended to exaggerate the discreteness of the ecotypic units. Böcher (1949), for instance, points out the difficulties involved in fitting the complicated data obtained from studies on *Prunella vulgaris* into discrete ecotypes. He appreciates the convenience of being able to single out the most representative character combination in a given locality and give it a name. But in that event "the reader must not be left in doubt that the name does not correspond to a well-defined unit." It is probable, he thinks, "that many of the plants which Turesson has cultivated will, on closer study, prove to behave like *Plantago maritima* or *Prunella vulgaris*," where the variation recorded is largely clinal. Current genecological work, indeed, suggests that the emphasis is increasingly being transferred from attempts to delimit ecotypes to the study of trends of ecotypic differentiation. This transfer of emphasis from the conceptual ecotype unit to the demonstrable reality of ecotypic differentiation in no way vitiates Turesson's concept.

It has been the custom, for obvious practical reasons, to apply the term *ecotype* to a population if any part of its heredity is demonstrably related to a particular feature of its environment. However, if an ecotype were to be established on the basis of a particular genotype–environmental relationship the same population might on the basis of other criteria again qualify for ecotype status with perhaps new boundaries. This is one of the reasons which make it so difficult to present a comprehensive picture of ecotypic differentiation in terms of territorially exclusive ecotypes. Moreover, because the characteristics which are most ecotypically responsive are so often quantitative ones, demonstrable ecotypic differentiation related to a particular environmental gradient is comparatively seldom discontinuously partitioned into discrete ecotypes, but, instead, it is commonly found to be arranged more or less continuously. In other words, ecotypic differentiation is commonly ecoclinal. The alternative to recording a few of the most discretely differentiated habitat populations as ecotypes is, therefore, to record whatever trends of ecotypic differentiation the investigator is able to detect by the techniques he employs.

At the present time, even the real significance of Turesson's concept is in danger of being masked by the loose way the term ecotype is sometimes used. Carter (1951), for example, refers to small populations which show geographical phenotypic differences resulting as responses to the direct action of environment, as ecotypes, and says (p. 141) "They are most noticeable in plants, but may occur in animals . . . . Variation of this type is non-genetic." Commonly, however, the term ecotype is

now used simply to denote a habitat population. Such wrongful usage has been commented on by Heywood (1959), who points out that the term ecotype is probably as much abused and misused as correctly employed in contemporary biology, and he is of the opinion that the misunderstanding which has grown up around this simple word is such that it sometimes appears that it would be better to abandon it and use the phrase ecotypic differentiation.

If ecotypic differentiation were always, or even generally, found to be associated with characteristic sets of correlated, taxonomically useful attributes, the recording of ecotypic patterns would present no novel problem. But it is largely because of the limitations of orthodox taxonomy at the micro-evolutionary level that a number of special classifications have been proposed for dealing with experimentally determined units. However, at this level the need is not so much for a hierarchal classification as for a series of category terms to which the micro-evolutionary units could be referred. It was with the object of providing such a series that the *deme* terminology was introduced (Gilmour and Gregor, 1939; Gilmour and Heslop-Harrison, 1954, and Gilmour, 1960). The essence of the deme terminology is the construction of a series of category terms by the addition of one or more virtually self-explanatory prefixes to a "neutral" suffix—*deme*. This suffix denotes any group of individuals of a specified taxon, and should never be used alone but always with a prefix, for instance, *gamodeme*, a deme composed of individuals so situated spatially and temporally that, within the limits of the breeding system, all can interbreed. Again, a *topodeme* is a deme occurring in a specified geographical area; an *ecodeme* one occurring in a specified kind of habitat; a *phenotopodeme* a topodeme differing from others phenotypically; and a *genoecodeme* an ecodeme differing from others genotypically. Now the term ecotype has been used in all these four senses, yet the categories have very different genecological significance. One of the merits of the deme terminology from the genecologist's point of view is that the categories are in no sense mutually exclusive, since the same population may be referable to several different categories according to which of its properties is taken into account, and the categories may be coincident or overlapping. Moreover, by avoiding absolute standards the adaptability of the terminology is such that it may be conveniently used in presenting the results obtained from a wide range of genecological techniques.

The present study, though it has entailed an examination of *Agrostis* and *Festuca* populations, is primarily concerned with the adaptive responses of *Plantago lanceolata* gamodemes to three floristically different types of phytosocial habitat on the one hand, and, on the other, to the relatively minor differences between sites within one of these types. While it is appreciated that adaptation probably always involves combinations of attributes, the following observations relate only to the variational patterns of individual morphologically simple characters chosen because of the ease and accuracy with which measurements could be made on "wild" as well as garden-grown material.

Observations

Samples were taken from ecodemes occupying the following three phytosocial types of habitat: A, species rich pasture; B, species poor vegetation lightly grazed; and C, *Molinia caerulea* dominated vegetation virtually ungrazed. Each of these three types of habitat was spatially fractionated into a number of representative sites scattered throughout a mosaic of different habitats mainly within an area of about 70×30 miles in southern Scotland. It is probable that with sites occurring in this manner any fortuitous genetic differences between site-populations would be randomly distributed throughout all three ecodemes. The aim was to sample a large

TABLE 1. *"Wild" and "garden" site-sample data: Means of site-sample means in respect of the phytosocial environments A, B, and C*

Type of habitat	No. of site-samples		Leaf length means (cm)		Stem length means (cm)	
			Plantago		*Agrostis*	
	P. lanceolata	*Agrostis*[1] agg.	wild	garden	wild	garden
A	41	53	6.2 ± 0.15	16.3 ± 0.10	24.8 ± 2.93	57.0 ± 0.10
B	13	37	6.7 ± 0.69	19.0 ± 0.24	30.6 ± 1.96	57.6 ± 0.15
C	6	25	28.1 ± 1.70	22.5 ± 0.45	47.4 ± 1.44	61.9 ± 0.26

[1] *Agrostis tenuis-canina* aggregate.

number of populations, and it was therefore necessary to restrict severely the size of samples taken. Eight plants of *Plantago lanceolata* were collected from each site-population along with eight plants of *Agrostis* and eight of *Festuca ovina*, and in order to avoid sampling the same genotype more than once the plants were taken at not less than ten-yard intervals. Because the site-samples were drawn from areas of such small extent, each *Plantago* sample has been regarded as representing a gamodeme. Leaf lengths of the plantain and stem lengths of the grass samples were measured, and then a small part of each plant, a single tiller in the case of the grasses, was over-wintered in an unheated glasshouse. (The reason for measuring the leaves of plantain rather than stems was because not every plant in a sample had stems. Subsequent garden tests showed the lengths of these two attributes to be positively and significantly correlated: $r = +0.88$). In the spring all plants were completely randomized in a garden trial, and data were collected during the following year. It is considered that the garden technique employed makes it legitimate to accept differences between groupings founded on garden data, and reaching statistical significance, as valid evidence of genotypic differentiation.

Table 1 summarizes the position as far as the average responses of the *Plantago* gamodemes to the major habitat differences are concerned. Given that the differences between the "garden" values can be accepted as proof of genotypic differentiation, it is clear that on the basis of the average response of its constituent gamodemes each ecodeme of the series A, B, and C is genotypically differentiated in respect of leaf length. Thus insofar as leaf variation is concerned the *Plantago* ecodemes A, B, and C are genoecodemes. It will also be seen that there is an evident parallelism between the "garden" values and average values for the phenotypic expressions of the three groups of gamodemes in the "wild." The pattern is very similar in the case of *Agrostis*. However, apart from the fact that the *Agrostis* aggregate comprises two hologamodemes that do not interchange genes with freedom, these two sets of data are not strictly comparable because although all the 60 *Plantago* sites contained *Agrostis*, 55 of the *Agrostis* sites lacked *Plantago*.

As already mentioned, the purpose of the investigation was to determine the responses of *Plantago lanceolata* gamodemes not only to major habitat differences but also to relatively very minor ones. Since a floristic definition of minor environmental differences proved impracticable, an attempt has been made to appreciate such differences by inference from the correlated responses of certain constituents common to all sites. Considering first the samples from the major habitats A, B, and C it is evident from the correlations (table 2) that the environments of the individual sites occasion corresponding responses in each of the common constituents, for instance, leaf length in *Plantago* and stem length in *Agrostis* and *Festuca* increased in unison from habitat A towards C. Thus it seems safe to assume that these differences in the

TABLE 2. *Correlations between "wild" site-sample means*

Habitat	No. of site-samples	Correlations	r	Significance
ABC	29	*P. lanceolata* leaf length *Agrostis* agg.[1] stem length	+ .88	.01
	14	*P. lanceolata* leaf length *F. ovina* stem length	+ .73	.01
	54	*Agrostis* agg. stem length *F. ovina* stem length	+ .86	.01
A only	10	*P. lanceolata* leaf length *A. tenuis* stem length	+ .86	.01
	20	*P. lanceolata* leaf length *Agrostis* agg. stem length	+ .92	.01

[1] *Agrostis tenuis-canina aggregate.*

"wild" leaf and stem length expressions are not just sporadically occurring differences, but are in fact controlled in a characteristic way by differences in the site-environments. In contrast to the wide range of site-environments covered by habitats A, B, and C, the range within type A alone is small. Yet the *Plantago* and *Agrostis* responses are once more highly correlated, from which it has been deduced that the distributions of the expressions of these responses are again environmentally controlled and, therefore, that the expressions themselves reflect minor site differences too indeterminate to allow of objective definition.

Turning now to the correlations between the "wild" and "garden" site-sample means (table 3), and first taking into account the total range of environments represented by habitats A, B, and C, it will be observed that the different *Plantago* leaf expressions exhibited by samples under the environments of their respective sites are significantly related to the differences exhibited by the same samples after the effects of the site-environments have been removed by the garden technique employed. Since

TABLE 3. *Correlations between "wild" and "garden" site-sample means*

Habitat	No. of site-samples	Correlations	r	Significance
ABC	27	*P. lanceolata* leaf length wild : garden	+ .53	.01
A only	21	*P. lanceolata* leaf length wild : garden	+ .01	N.S.
ABC	46	*Agrostis* (agg.) stem length wild : garden	+ .62	.01
A only	17	*Agrostis* agg. stem length wild : garden	+ .44	N.S.
ABC	29	*P. lanceolata* leaf length (garden): *Agrostis* (agg.) stem length (wild)	+ .50	.01
A only	14	*P. lanceolata* leaf length (garden): *Agrostis* agg. stem length (wild)	+ .01	N.S.
M[1]	10	*P. maritima* leaf length wild : garden	+ .59	.05

[1] A mountain grassland type of habitat.

the environments of habitats A, B, and C occasion characteristic leaf expressions in *Plantago* (table 2), and since, as the first coefficient in table 3 reveals, corresponding characteristics differentiate the garden samples, it can be assumed, even in the inevitable absence of positive proof, that the *Plantago* leaf length genoecodemes A, B, and C are essentially products of environmental selection. If this is a correct conclusion, and there seems no reason to doubt it, then it follows that the expressions of leaf length under the "wild" conditions must have some adaptive significance. Although the term genoecodeme does not carry the implication of origin associated with Turesson's ecotype, the foregoing evidence provides reasonable grounds for regarding these leaf length genoecodemes as ecotypically differentiated assemblages. If now the *Plantago* gamodemes of habitat A only are considered, the correlation between the "wild" and "garden" leaf length expressions is not significant despite the evidence provided by the correlations (table 2) that the sites of this habitat differed ecologically to the extent of affecting not only the expression of leaf length in *Plantago* but also of stem length of two associated community constituents. Thus the *Plantago* gamodemes occurring within this A type of habitat represent a series of leaf length plasmoecodemes, for while they differ from each other phenotypically, apparently they are not genotypically differentiated.

By way of contrast the correlation (table 3) between "wild" and "garden" measurements in respect of the gamodeme samples of the mountain ecodeme, M, of *Plantago maritima* just attains statistical significance. In this case, however, habitat M, subsuming a variety of upland sites, is more loosely defined than any one of the other three types mentioned in the table, and consequently the range of phytosocial environments in this habitat more closely approaches that of the entire gradient ABC than the range within habitat A alone.

Reflections

That the actual expressions of leaf length exhibited by the *Plantago* gamodemes of the genoecodemes A, B, and C resulting from the phenotypic responses to the prevailing habitat conditions must have some adaptive significance has already been suggested. Again, it seems justifiable from the data presented in table 2 to assume that the differences in phenotypic expression between the *Plantago* gamodemes of ecodeme A alone also have adaptive significance. If this is a valid inference, then the statistically insignificant correlations between the "wild" and "garden" measurements (table 3) in respect of the gamodemes within habitat A suggest that when the differences between habitat environments are relatively minor, phenotypic plasticity has the effect of cushioning or shielding genotypes against the full impact of environmental pressures. Certainly, judging from the present results, it would seem to require a selective pressure of considerable intensity before the manifestations of exogenous adaptation become detectably associated with ecotypic adaptation, though as Frankel (1959) has pointed out "the nature of adaptation is determined not, as is often assumed, by the relative severity of [environmental] stress, but by the nature and extent of variation" of that stress. However, it may well be a cryptic feature of ecotypic selection to favor genotypes with a high plasticity potential. In this connection it is of interest to note that in *Potentilla gracilis* Clausen, Keck, and Hiesey (1940) showed that "the capacity for modification varies from one plant to another even within a population or an ecotype."

On the other hand, the fact remains that when a phenotypic response happens to be of adaptive significance it is not infrequently accompanied by an apparent genotypic response. Waddington has emphasized that "natural selection for the ability to develop adaptively in relation to the environment will build up an epigenetic landscape which in its turn guides the pheno-

typic effects of the mutations available" (1957, p. 188). In other words, if a phenotype, valuable in a selective sense, happens to arise as a direct response to the prevailing environmental conditions it may ultimately achieve genetic fixity as a result of the operation of selection acting in the same direction over a number of generations, but only insofar as the requisite genes already exist in the original population. Thus it is not strictly correct to regard the environment merely as a phenotypic sieve. In addition, it is something which "builds into the epigenetic system tendencies to be easily modifiable in ways which are adaptive."

Though ecotypic adaptation is doubtless a common phenomenon, it is by no means always easy to demonstrate, for only the prevailing habitat conditions are available to the investigator. Yet the characteristics of populations of perennial plants may reflect the pressures of past rather than present environments and thus current relationships between hereditary attributes and habitat factors may be blurred or even obscured. Harberd (1958, and unpublished) has produced fairly conclusive evidence of a single genotype of *Festuca rubra* having an interrupted spread over a distance of 240 yards. Under the favorable competition-free conditions of the experimental garden this particular genotype increased in diameter at the rate of 18 inches per annum, so at a conservative estimate such a spread suggests that it originally succeeded in establishing itself about four hundred years ago. It is possible that the continued success of "relict" adapted genotypes of this kind reflect remarkable capacities for phenotypic adjustment to environmental changes.

Under the ranges of environment obtaining in Britain it seems to be the exception rather than the rule to find ecotypic variation discretely fractioned. In certain circumstances it does, as, for instance, where lead mine spoil-heaps occur, lead tolerant and lead susceptible genoecodemes apparently do not intergrade (Jowett, 1958; Wilkins, 1960). On the other hand, the studies of Clausen, Keck, and Hiesey (1940) indicated that ecotypic discontinuities might be found more commonly in California. It is true that with two exceptions the Californian ecotypes of *Potentilla glandulosa* described by them at that time were discretely distinct. They differed from each other in many characters both physiological and morphological and were sufficiently recognizable to be accorded taxonomic rank as subspecies; some of their diagnostic features indeed appearing to have more taxonomic than ecotypic significance. Since then, however, the *Potentilla glandulosa* situation has turned out to be more ecotypically complex than the 1940 results suggested, as is made clear by Clausen and Hiesey (1958). They now find that "In *Potentilla glandulosa* there appears to be both continuous and discontinuous variation. A complete intergradation, or cline, occurs for one character [time of flowering] within one sub-species, whereas other sub-species appear to show random variation for the same character. Further study would probably multiply such examples of degrees of discontinuity and degrees of intergradation among the various ranks of natural entities." As far as the ecotypic characteristics are concerned these taxonomic subspecies, though internally ecotypically complex, do seem, in a broad way, to represent discretely ecotypically differentiated entities, but perhaps rather less discretely than previously thought.

Sometimes ecotypic units will coincide with recognized taxa, but more often the genecologist will be faced with a situation not unlike that which obtains within the subspecies of *Potentilla glandulosa,* and the chances are that very few characters, perhaps only a single character, will show variation bearing a relationship to changes in the chosen environmental factor, or group of factors. Since, however, selection in populations is based on character combination and not on single characters, it must be acknowledged that "too much

emphasis on gradients in single characters may serve to confuse rather than clarify the whole pattern of variation" (Stebbins, 1950). Nevertheless, when contemplating a genecological study there is, for practical reasons, virtually no escape from subjectively abstracting from the habitat complex of factors a few of the more obvious ones, and determining whether they can be shown either collectively or singly to affect the variation distribution of any character or characters, always remembering that factors other than those chosen may influence the variational patterns observed. An example from experiments with *Plantago maritima* illustrates this latter point. In this case attention was limited to a readily appreciated range of edaphic-phytosocial habitat conditions. From garden data it was possible to show that gamodeme samples collected from three recognizably different types of habitat on the Scottish mainland represented three ecoclinal genoecodemes on the basis of leaf and stem length and growth-habit variation. Samples collected on one of the Atlantic islands off the Scottish coast from types of habitat corresponding to those of the mainland could on the same criteria also be arranged in the same ecoclinal sequence. But because the stem and leaf lengths of the island topodeme were in general shorter, the two ecoclinal series of genoecodemes, though parallel, were not coincident; a pattern which reflected the influence of some other factor, perhaps climate, which had not been taken into account.

Unfortunately, ecotypically significant characters of the kind just mentioned are of such a nature that, strictly speaking, the results of sample comparisons in any garden experiment are unique to that experiment, and as Clausen, Keck, and Hiesey (1940) remark "ecotypical differences may be accentuated at one station over another and the most vivid impressions of those differences are obtained at the garden where the species as a whole does its best." This is a situation for which the deme terminology caters, as any implication of absolute standards of difference is rigidly excluded.

Thus while the numerical descriptions of the *Plantago lanceolata* genoecodeme given in table 1 are not presumed to be universally diagnostic, they do reveal a pattern of ecotypic differentiation which in fact exists and is consequently worth recording, but in applying the deme terminology to these or other micro-evolutionary units of variation it is always necessary to remember that no deme category as such bears a direct relationship to any category of orthodox taxonomy.

Summary

1. Observations on leaf length responses of *Plantago lanceolata* gamodemes to a range of habitat conditions indicate that it requires a considerable difference in phytosocial environment before exogenous adaptation becomes associated with detectable ecotypic differentiation.

2. Since ecotypic discontinuities are rarer than had initially been imagined, it would seem that a better understanding of ecotypic patterns could be achieved through the accumulation of records in which the emphasis has been transferred from the discrete ecotype to the trends of ecotypic differentiation. Such a change in emphasis would in no way invalidate Turesson's original concept. What does detract from its significance is the wrongful use of the term ecotype.

3. To facilitate the accumulation of information relating to ecotypic as well as to other micro-evolutionary patterns, it is suggested that the investigator should record in terms of demes whatever entities he is able to detect by the techniques he chooses to employ.

4. A merit of the deme terminology from the genecological point of view is that the categories are in no sense mutually exclusive, since the same population may be referable to several different categories according to which of its properties is taken into account, and the categories may be coincident or overlapping. Moreover, by

avoiding absolute standards the flexibility of this terminology makes it useful over a wide range of genecological techniques.

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COWBIRD PARASITISM AND THE POPULATION OF THE KIRTLAND'S WARBLER

HAROLD MAYFIELD
River Road RFD, Waterville, Ohio

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The geographic range of a species and the abundance of the species within the geographic range are determined by a large number of interacting environmental factors. The analysis of these factors is particularly difficult in birds, which as warm-blooded animals are less dependent on the environment than ectothermal animals, and as highly mobile creatures can escape seasonal changes in climate by migration. The relative importance of certain physical and biotic components of the environment can be studied best in those species which are currently showing a conspicuous increase or decrease in numbers or in size of geographic range. A bird species that appears to be undergoing a rapid decline in numbers at the present time is the Kirtland's Warbler, *Dendroica kirtlandii*.

GEOGRAPHIC RANGE AND ANNUAL MIGRATIONS

The Kirtland's Warbler nests only in particular localities within an area less than 100 miles square in northern Lower Michigan (fig. 1). It spends the rest of the year in the Bahama Islands. Northbound migrants enter the United States in April, and the first of them reach the nesting ground about the middle of May. They begin to leave the nesting ground in late August and nearly all have departed by September 15. The scanty records of spring and fall migrants are mostly in a direct path between Michigan and the Bahamas.

The comparatively barren conditions of its summer and winter homes suggest it is a relict species (Amadon, 1953). Presumably unable to survive the pressures from other life in richer habitats, it has found a niche for existence in the comparative sanctuary of habitats that are marginal for most other creatures.

BREEDING HABITAT

The Kirtland's Warbler breeds in areas which provide habitat conditions as follows: Extensive tracts of small coniferous trees crowded densely enough in places for the branches to interlace but spaced widely enough in other places to provide generous openings; the trees bearing live branches close to the ground; the ground cover short but tall enough to conceal the nest, which is placed on or imbedded in the sand; the soil dry and porous.

This situation occurs in the northeastern coniferous forest only on sandy uplands that have been scoured again and again by forest fire. The only tree growing naturally on such areas and providing the required configuration is the jack pine, *Pinus banksiana*. Consequently, almost from the day of discovery of its nesting ground in 1903, this bird has been regarded as the jack pine warbler, although forestry plantings in recent years have shown that other pines are equally suitable if the basic conditions are reproduced.

Recent History of the Breeding Habitat and Population.—The necessary conditions for the nesting of this bird probably have not existed in recent times anywhere except near the southern limit of the jack pine in northern Lower Michigan, where there is a considerable region with uniform soil conditions and few barriers to forest fire, that is, very few lakes, swamps, or intrusions of hardwoods. Although the jack pine ranges from Nova Scotia to British Columbia and northward almost to treeline, and although there are dry pinelands of considerable extent at similar latitudes in Minnesota, Wisconsin, and Ontario, I doubt that any other areas have been fully suitable in recent times. Perhaps briefly after forest fire a good many square miles

FIG. 1. Breeding range of the Kirtland's Warbler (black dot) within the range of the jack pine in North America (shaded).

have been temporarily suitable, but I doubt such areas were sufficiently persistent, extensive, and compact to support a population of these warblers elsewhere.

In this region up to modern times forest fires started by lightning or by man, unimpeded by natural barriers, must have burned tens of thousands of acres at a time. At the time the white man arrived there were probably less than 500,000 acres of jack pine in the Lower Peninsula (Zimmerman, 1956: 31), of which perhaps 10 per cent, say 50,000 acres, were in the stage of growth suitable for the Kirtland's Warbler at all times. Many of these tracts were peripheral and otherwise unsuitable, however, and I doubt if the average population of Kirtland's Warblers was more than 5,000 birds.

Then, about 1875, the lumbering of the interior pinelands of Michigan began. Slashings were allowed to lie where they fell and fires, particularly in late summer and fall, swept fiercely through the region. Not only was there no attempt to stop them, but sometimes they were started willfully to clear the land for farming and to encourage the blueberry crop. Eye witnesses have left vivid accounts of seasons never free of the pungent odor of fire, with a pall of smoke clouding the sky for hundreds of miles down wind. In the period from 1885 to 1900, the time of greatest lumbering and greatest fires, I suspect the area suitable for Kirtland's Warblers may have been as much as 200,000 acres, several times what it had been before the white man. At this time also the right habitat occurred in large tracts, which are probably favorable to the bird. The population of warblers seems to have risen accordingly. For example, the number of specimens of the Kirtland's Warblers taken by collectors on the wintering ground were as follows:

1860s and earlier	0
1870s	1
1880s	44
1890s	22
1900s	2
1910s	2
1920s and since	0

Since the days of large-scale lumbering, the *extent* of fires has been greatly reduced,

first by the clearings made by man, and more recently by organized firefighting. However, the increased human population in the region has brought a great many accidental fires and much planting of jack pine; so I suspect that the total area in small jack pines in the last 40 years has averaged about 100,000 acres, about twice as much as before the white man arrived but mostly in smaller tracts, which I believe to be less favorable to the warblers.

In 1951, a count of singing males indicated that the total population of males and females of this bird was less than 1,000 (Mayfield, 1953). The total of the areas occupied by 432 pairs actually counted at that time was about 12,000 acres, about one-eighth of the region's total acreage in the right stage of tree growth. In fact, at no time since the first nest was discovered has the Kirtland's Warbler used more than a fraction of the apparently suitable habitat in its nesting region.

Factors Restricting the Bird to a Narrow Range.—This bird has some characteristics which, although seeming to have survival value, tend to limit its breeding range to a compact central homeland. With these characteristics the bird is ill-equipped to pioneer new and distant areas: (a) the bird has excellent homing abilities, as demonstrated by its long migration between small winter and summer ranges; and (b) it has a tendency—or perhaps necessity—to breed in colonies.

As a result, this bird, returning precisely to a spot on its ancestral nesting ground and finding it no longer suitable, may wander about and readily discover another within a few miles, say 25 miles, but not at a great distance, say 100 miles or more. An individual or two may stray farther, but not a sufficient group to establish a distant colony.

Effect of the Cowbird

A major factor in the life of the Kirtland's Warbler is the social parasite, the Brown-headed Cowbird, *Molothrus ater*. The cowbird removes eggs from the nest of the warbler and lays its own in their place, and the warbler cares for the foreign eggs and young as though they were its own, at a severe cost in production of warblers.

The Kirtland's Warbler is heavily parasitized, and it suffers at every step in the nesting process; eggs to be incubated, eggs hatching among those present, and nestlings fledged among those hatched.

In Kirtland's Warbler nests, I find cowbirds remove slightly more host eggs than they lay of their own. (This is a higher loss per cowbird egg laid than reported for any other host species.) Next, the mere presence of cowbird eggs reduces the hatching rate of warbler eggs in the same nest. (Presumably this is because the larger diameter of the cowbird eggs reduces the heat available to the warbler eggs.) And finally, the young cowbirds trample to death many nestling warblers, particularly in the first two days of life. Typically, the young warbler weighing 1.5 g hatches in the presence of one or more cowbirds weighing 10 g each. If two or more cowbirds hatch ahead of the warblers, the result is death to all the warblers. I have never found a young warbler more than four days old in the presence of two or more older cowbirds, and the presence of even one cowbird reduces by one-half the chances of hatched warblers' fledging.

For analysis of the extent of cowbird parasitism among Kirtland's Warbler nests, I restrict my sample to nests seen with eggs (ignoring nests found after the eggs had hatched) and seen on more than one day (to be certain the clutches were complete). In this sample of 142 nests, 75 nests or 55% contained cowbird eggs. The numbers of eggs in these nests also permit us to calculate the number of warbler eggs removed by the cowbirds, as follows:

Kirtland's Warbler Egg Losses Through Removal of Eggs by Cowbirds

	Parasitized	Not parasitized
Nests	75	67
Warbler eggs	205	310
Warbler eggs per nest	2.75	4.63
Cowbird eggs	125	0
Cowbird eggs per nest	1.67	0

Warbler eggs removed per parasitized nest, $4.63 - 2.73 = 1.90$

Proportion of warbler eggs removed in parasitized nests, $\frac{1.90}{4.63} = 0.41$

Further analysis of nests available to me at various stages in the nesting process, the full data and calculations not given here (Mayfield, 1960), leads to a summary of losses from this cause, as follows:

Kirtland's Warbler Losses from Cowbirds

(In nests that continue to fledging; losses expressed as per cent of eggs laid)

	In parasitized nests	In all nests, of which 55% were parasitized
Eggs removed by cowbirds	41	23
Hatching loss attributed to presence of cowbird eggs	6	3
Nestling losses attributed to presence of cowbird nestlings	31	17
Total	78	43

This table, which shows the cowbird pressure exerted proportionately at each nesting stage, somewhat exaggerates the total effect of the cowbird on the warbler. It gives the effect of the cowbird in nests that continue all the way to fledging and ignores the fact that a great many nests are destroyed or deserted (usually from causes unknown). In connection with this table, the consequence of destruction and desertion of nests is to reduce the number of eggs present at hatching time and the number of nestlings exposed to cowbirds, and, hence, to reduce the proportions lost at hatching and in the nestling stage as a percentage of eggs laid.

However, when nests are destroyed or deserted, the loss is not what it appears to be, since the Kirtland's Warbler promptly builds again unless the season is too far advanced. On the other hand, when the warbler succeeds in producing young to the point of fledging, it does not nest again in the same season. The cowbird pressure continues throughout the successive nestings. Thus, the result of the destruction or desertion of nests is usually delay rather than net loss, except that clutches are usually smaller in later attempts after the first of the season.

Therefore, to see the cowbird damage in proper perspective, we turn to a consideration of the annual yield in fledglings per pair of adults. In my study I have found this to be 1.4 per year. On the other hand, it is 2.2 per year without cowbird interference (Mayfield, 1960). Thus, the production of fledglings is reduced about 36 per cent by the work of cowbirds.

Some birds might easily sustain this rate of loss, but not the Kirtland's Warbler. The survival rate of adults from one June to the next is 60 per cent. Therefore, with a production of fledglings at a rate of 1.4 per pair, the survival rate of first-year birds would need to be 57 per cent in order to replace the adult losses and maintain a stationary population. This survival rate of young birds from fledging to the next year's nesting season is scarcely believable. On the other hand, without cowbird interference, the production and survival rate of adult warblers would require a survival rate among first-year birds of 36 per cent, a fairly high but perhaps attainable figure.

The count of Kirtland's Warblers taken in 1951 is to be repeated in 1961 to measure the 10-year trend.

The production of fledglings per year by a pair of adults has been found to range from 1.7 to 8 in several other songbirds (Lack, 1954). In the Ovenbird, another American ground-nesting warbler, it was 2.9 (Hann, 1937). In the Prothonotary Warbler, which nests in tree cavities, it was 1.5 (Walkinshaw, 1953); but significantly, the last figure was observed at the extreme limit of the range of a widespread species and this peripheral population may be replenished from a richer yield in the main part of the range.

Special Vulnerability of the Kirtland's Warbler.—Other birds studied have found partial solutions to the cowbird problem

in various ways; they eject the foreign egg or build a new nest floor over it; they desert when molested and start again in another site; they rear their young successfully in spite of cowbirds in the nest; they continue nesting late in the season after the cowbird pressure has abated; or, like the Red-eyed Vireo, they nest also in other regions outside the range of the cowbird. However, the Kirtland's Warbler has none of these defenses. It normally rears only one brood a year and does so in a short nesting season that is completely bracketed by the egg-laying season of the cowbird. Further, it has the severe disadvantage of an incubation period a day or two longer than that of most other small songbirds, allowing young cowbirds two days head-start over the warbler nestlings.

This is an unusual type of pressure by one species upon another, for, unlike ordinary forms of predation, here a reduction in the "prey" species does not reduce the pressure. The cowbird is not dependent on the Kirtland's Warbler and does not specialize on it, parasitizing many other songbirds at the same time in the same areas.

The Recent Arrival of the Cowbird.—The cowbird probably has been a significant factor in the existence of the Kirtland's Warbler only in modern times—perhaps since 1890. Here are the circumstances that point to this conclusion.

Friedmann (1929) in his study of the cowbirds expressed the view that the Brown-headed Cowbird was originally a bird of the Central Plains, where it was associated with the large grazing animals, particularly the bison, and that it was rare or missing in the eastern forests before the settlers arrived with their grazing stock. This relation between the farmer and the cowbird seems borne out by other evidence also, some of it recent. Norris (1947) in Western Pennsylvania, an area with much farmland, found the Red-eyed Vireo to be the most heavily parasitized of all the species he studied. Similarly, Southern (1958), working in the northern part of the Lower Peninsula of Michigan, with many farms nearby, found 75%, a remarkably high ratio, of Red-eyed Vireo nests parasitized. In contrast, Lawrence (1953) in a part of Central Ontario largely forested, found no parasitism at all among 44 vireo nests.

Historically, we have some evidence from adjacent regions. Fothergill, an active field worker in southern Ontario whose meticulous field notes for the period 1817–1840 are now in the Royal Ontario Museum at Toronto, saw no cowbirds at all. Similarly, in Ohio, Kirtland (1838) placed the cowbird in the first check-list of the state "on doubtful authority," but Wheaton (1882) quoted Read as saying in 1853 it had "recently increased in numbers," and in 1864 Kirtland wrote in his notes "abundant, formerly rare" (Christy, 1936). Thus, in Ohio the cowbird seems to have progressed in about 20 years from "rare" to "abundant," a period that coincided closely with the opening of the forest to farmland in that state.

In Michigan until the mid-1800's, virtually the entire human population was limited to the first four tiers of counties along the southern border. The rest of the state was nearly unbroken forest.

Although the semi-open land inhabited by the Kirtland's Warbler may have been locally suitable for the cowbird, although lacking any great number of grazing animals, it was separated by almost 200 miles of unbroken forest from the more extreme outposts of the prairie peninsula that reached up from the Central grasslands as far as the southwest corner of Michigan. Also, the prairies in southwestern Michigan were moist, grown high in summer with towering grasses and probably unsuitable to the cowbird. So I believe there were no cowbirds in Kirtland's Warbler country before the white man arrived.

The cowbird came north with the settler, who reproduced with patches of fields and domestic animals the conditions of the western short-grass plains. These settlers reached the pinelands with the lumber-

men; in fact, on the poorest soils farming was at its height when the marginal farmer had an outlet for his products near at hand in the lumber camps, and many of these farms were abandoned soon after the lumbermen departed. Therefore, I conclude the cowbird arrived in the Kirtland's Warbler range in the 1870's and within perhaps 20 years, that is, by about 1895, built up to abundance. This would have been several years before the discovery of the nesting ground of the Kirtland's Warbler.

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SUMMARY

The Kirtland's Warbler, a rare species of bird with a small and special habitat, is in a precarious position for survival. For habitat in its nesting range, it is dependent on forest fires, which are no longer as extensive as in former times and therefore perhaps do not provide many areas that are optimal for this bird. Now, with a reproductive potential that is marginal, the Kirtland's Warbler is subjected to a new and unrelenting pressure from the social parasite, the Brown-headed Cowbird. Against this threat it has developed no defense.

The cowbird takes a toll of the warbler at every stage of the nesting process and reduces the production of warbler fledglings about 36 per cent below the level achieved in the absence of cowbirds. This toll might not be serious for a more prolific species but may prove to be crucial to the Kirtland's Warbler, since there is no promise that the pressure will be relieved even though the warbler approaches the brink of extinction.

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THE ADAPTIVE SIGNIFICANCES OF INTRASPECIFIC TRENDS OF VARIATION IN WING LENGTH AND BODY SIZE AMONG BIRD SPECIES

T. H. HAMILTON
Harvard Biological Laboratories

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This paper describes the various trends of clinal variation in wing length (usually thought to be an indicator of body size; see Amadon, 1943) in continental bird species having wide distributions. Attempts are made to evaluate the several hypotheses put forward by various workers as possible explanations for the actions and interactions of the selection forces apparently responsible for these trends.

Recently there has been a renewed interest in the old, and still unresolved, evolutionary problem of the adaptive meanings of intraspecific variation in body size. This interest in the adaptation of homeothermic animals at the population or intraspecific level has been shown by two groups of workers differing in methodology of approach: adaptation physiologists (e.g., Scholander, et al., 1950a, 1950b; Scholander, 1955, 1956; Irving, 1957, 1960) studying heat conservation and dissipation in individuals of mammalian and avian species; and population evolutionists (e.g., Traylor, 1950; Snow, 1954; Salomonsen, 1955; Mayr, 1956; Moreau, 1957; Hamilton, 1958) studying intraspecific variations of biological characters of wide-ranging species of birds. The former group, rediscovering the long-known rule that body insulation is relatively greater in homeothermic individuals of colder regions (see, e.g., Rensch, 1939a), have criticized the old concept that heat conservation is one of the physiological principles underlying Bergmann's rule, and have gone so far as to deny the validity of the ecogeographical rules. Starting from the studies and summaries of Rand (1936), Huxley (1939, 1942), Rensch (1939a), Miller (1941), Mayr (1942), and Pitelka (1951)[1], the evolutionists, working with population samples, have concentrated on determining the statistical validity and degree of expression of ecogeographic variation. Secondarily they have speculated on the factors responsible for the often noticeable correlations between biological character clines and gradients of the environment.

Geographic variations of biological characters such as wing, bill, and tail lengths are generally not random, but orderly, for wide-ranging, continuously distributed, sedentary species. It follows then that selection forces—admittedly operating through unknown mechanisms—are responsible for the non-random nature of ecogeographic variation. A corollary of this statement is that the characters involved are genotypic adjustments to geographically differential selection pressures. However, whether the biological characters manifested as clines are genotypic or phenotypic is not a problem that necessarily strikes at the foundation of the theory of ecogeographic adaptation. As will be discussed below (p. 191), the theory of initial phenotypic adjustments followed by genotypic control is one explanation (the Baldwin effect) for the occurrence of climatic adaptation at the intraspecific level.

Much of the confusion concerning the interpretation of ecogeographical rules stems from a misunderstanding of the

[1] The evidence for ecogeographical rules for birds and mammals in general is so extensive that further citation here is unnecessary. That rules such as Bergmann's and Allen's are empirically apparent even for historically sedentary segments of *Homo sapiens* has been continuously documented by Schreider (e.g., 1950, 1951, 1957). It is also of interest here that these rules may be valid for some poikilothermic species (Ray, 1960).

nature of variation in wing length. Wing length is used as an indicator of body size only because weights of specimens are generally lacking, and it is quite obvious that bone measurements are needed for critical evaluations. Even though an increase in body size seems to result in some increase in wing length and area (see, e.g., data of von Lendenfeld, 1905), there is always the possibility that certain selection forces will act directly to modify the structure of the wing. Thus, intraspecific variation in the length of the wing would seem to be interdetermined by (a) selection forces that only secondarily influence the length of the wing by increasing or decreasing general body size (an allometric effect?) and (b) selection forces that have an immediate effect on the wing. Accordingly, the net adaptive response of the wing to selection pressure may represent the combined operations of several selection forces that augment, counteract, or override one another in operation. For the present discussion, I make a hypothetical distinction between the expressions selection pressure and selection force. The former expression refers to the resulting operation at the population level of one or more selection forces. Thus, the latter represent components or vectors of the selective influence of the environment (*sensu lato*).

Factors Postulated as Exerting Selective Influences on Length of the Bird Wing

A. Selection Forces Operating Indirectly via Adjustments in General Body Size

1. Temperature.—Correlations between northward clinal increases in wing length and decreases in environmental temperature have long been known from intraspecific comparisons among sedentary Holarctic bird species of wide distribution (Bergmann, 1847; Allen, 1871, 1906). It is generally assumed that increased wing length in the colder regions of continents is a reflection of an increase in general body size, correlated with the severity of winter temperature (Rensch, 1939b; Mayr, 1942; Huxley, 1942). Snow's investigation (1954) of this ecogeographical rule clearly confirms the validity of the rule for some species of *Parus* (for *P. major*, see fig. 1). He states that, for available weights, wing length is a satisfactory indication of size in this genus, which consists of species that are primarily sedentary.

The intraspecific increase in size correlated inversely with environmental temperature is termed Bergmann's rule, and the references cited above list numerous examples. For example, Rensch (1939b) found that clinal increases in wing length of *Parus montanus* in the western Palearctic parallel decreasing winter isothermic values northeast into the north-central interior of Eurasia. In 1952, Lunk, studying the North American counterparts of *P. montanus*, found that the two allopatric species, *P. atricapillus* and *P. carolinesis*, together show a south to north cline for increase in wing length, corresponding to the progressive lowering of winter temperatures.

Various workers have postulated that the increase in general body size in colder regions is an adaptive modification for heat economy. They reason that, with increase in volume and relative decrease in exposed surface, heat loss to environment will be reduced, thereby facilitating heat economy during the colder seasons. Scholander (1955) criticized this theory, pointing out that northern species of mammals appear to have solved the problem of existence in colder regions by increases in body insulation, rather than by slight increases in body size. However, Mayr (1956) warned that such observation as Scholander's do not disprove the commonly accepted basis of Bergmann's rule, and, further, that climatic adaptation would be expected to occur as an *additive*, adaptive response, with opportunity for more than one selection force to contribute to the process. I have elsewhere (Hamilton, 1958) suggested that if Scholander's conclusions were entirely valid, we would "expect similar body sizes for in-

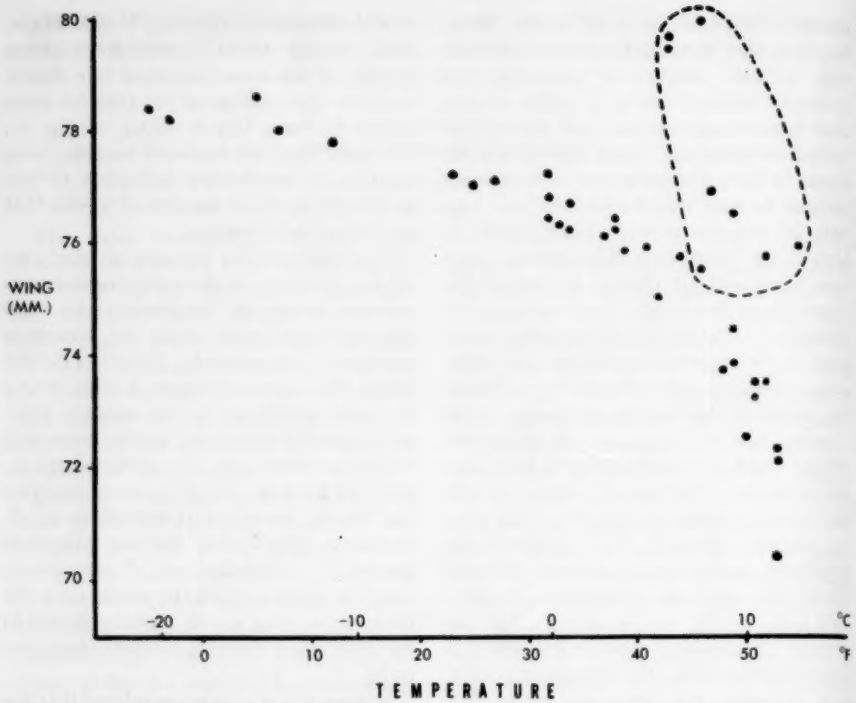

FIG. 1. Relation between wing length and the mean temperature of the coldest month for 39 populations of the Great Tit (*Parus major, major* segment) from the western Palearctic. The North African populations and one from southern Persia are enclosed by the broken line. Reproduced with the author's permission from Snow, 1954.

dividuals from widely separated localities within the species' range, other conditions being equal" — a situation not demonstrated by the references cited above.

2. Length of Foraging Day. — Snow (1954) noted that the variation of some populations of several species of *Parus* is an apparent exception to Bergmann's rule. He found that individuals of populations endemic to the higher altitudes of mountains of North Africa and the Near East tend to be larger than their conspecific counterparts subjected to similar winter temperatures in the higher latitudes of Europe. This latitude "effect," as he terms it, is shown for *Parus major* in figure 1, and it is readily seen that the populations cited above show noticeable departures from the expected clinal variation in wing length in relation to winter temperature.

Snow suggests that in the higher latitudes the short feeding day during the height of winter is a critical factor limiting body size. He reports, for example, that individuals of *P. major* in Scandinavia may be limited to daily food-gathering periods of 5–5½ hours, whereas in North Africa, conspecific individuals, during the briefest day of winter, are never restricted to less than 9 hours of foraging activity. The point here, he states, is that, other things equal, a larger body requires more food per day than a smaller body, and thus a larger body size might be "permitted" in lower latitudes.

There are two important points to Snow's theory of a latitude "effect." First, that the phenomenon is superimposed on the operation of Bergmann's rule is apparent for *P. major* in figure 1. Since the

variation of wing length in relation to winter temperature indicates a gradual leveling-off at higher latitudes, it would appear, as he states, that at higher latitudes some environmental factor is acting to inhibit or suppress the operation of Bergmann's rule. Second, in relating length of feeding day to body size, Snow presents a possible explanation to the question (raised by Scholander, 1955) of why birds, for intraspecific comparisons, are not even greater in size in extremely cold, continental regions. It is quite possible that selection for increases in body size, beyond a certain point, could tend to exceed the limits for which populations can effectively meet necessary energy requirements in a stringent environment. Thus a brief feeding day in winter may operate in the arctic latitudes to suppress increases in body size. In such a case of apparent conflict between opposing selection forces, a compromise adaptive response would be expected. And, accordingly, if adaptation for efficient heat conservation via shifts in body size were curtailed, then other devices or potential means for heat conservation might be strengthened or enhanced (see p. 189).

The evidence for the latitude "effect" is based only on five species of *Parus*. However, tremendous sampling of populations is necessary for demonstration of the leveling-off of a northern cline for increase in size, and there is still the problem of determining if the leveling-off is true for body weights or bone measurements as well as for wing length. For evaluation of the latitude "effect" it would be useful to have more information on clinal variation in body size within wide-ranging, sedentary species that occur both in temperate and arctic latitudes. For example, the ptarmigan species of the genus *Lagopus* provide a "natural experiment" for testing the reality of this "effect," and Koskimies (1958) has reported from Finland a northern cline for decrease in body weight for *Tetrao urogallus*. Whether this is an example of Snow's latitude "effect" remains to be shown.

3. Relative Humidity.—In a previous paper I presented some data that I interpreted (Hamilton, 1958) as indicating intraspecific correlations between (i) aridity and increased body size and (ii) humidity and decreased body size. These tentative conclusions were based for the most part on studies of geographical variation of wing length in some New World species. For example, in three species of the genus, *Vireo*, the populations resident to Middle America may be either larger (Sonoran region of Mexico) or smaller (eastern regions of Central America) in wing length compared conspecificly to migratory populations (for *V. solitarius* and *V. gilvus*) or to sedentary populations (for *V. huttoni*) breeding in the higher latitudes of North America. For two other species of this genus (*V. griseus* and *V. olivaceus*), it is definite that decreased wing lengths are characteristic of populations resident to warm and humid regions of the lower latitudes. For some other examples of these two intraspecific trends of variation in wing length, see my 1958 paper.

I now refer to this as a relative humidity response, manifested as either an aridity "effect" or a warm-humidity "effect" for, respectively, increases or decreases in wing length (and presumably body size) in the lower latitudes. In an attempt to gather data on intraspecific variation in body weight as a means of checking the reality of these "effects," I have found (Hamilton, MS) that evidence in support of the aridity "effect" is sparse and inconclusive.[2] However, the evidence for intraspecific decrease in body size in warm, humid regions of lower latitudes seems even stronger than originally reported. My survey of the literature on avian intraspecific variation reveals—for many widespread, sedentary

[2] For example, in my 1958 report, I demonstrated for the cardinal, *Richmondena cardinalis*, a southwestern trend for increase in length of wing going into the Sonoran region of Mexico. A survey of variation in body weight for this species reveals, however, an opposite trend with populations of northern and northeastern United States having the greatest body weights.

species—tendencies for clinal decreases in size or wing length into warm, humid continental regions. It would appear that previously such cases have been dismissed as demonstrating the "lack of operation of Bergmann's rule." However, there is a difficulty here; size decrease might also be explained by the increased density of tropical vegetation (see p. 185) or by the increased numbers of ecologically related, competing species (see below).

The reality of the two "effects" remains to be ascertained. The previous literature is not much help. Rensch (1931) pointed out that tropical races tend to be smaller than those of temperate regions, and cited *Parus major* as an example. Salomonsen (1955) reported (for *Falco columbarius*, *Guiraca caerulea*, and *Agelaius phoeniceus*) an intraspecific increase in size in Sonoran Mexico and the Great Plains of North America. Ripley (1950) apparently was the first to note that birds of arid regions tend to be larger than those of humid regions, and Etchecopar and Hüe (1957) cite this as Ripley's rule.

I have speculated (Hamilton, 1958) that the increase in wing length in warm, arid regions is an indication of size increases that facilitate conservation of metabolic water, and that the decrease of wing length in warm, humid regions is indicative of size decreases that facilitate effective heat dissipation. Relating to this, Bartholomew and Dawson (1954) find, in a variety of desert species, that for "unknown reasons" bird species or individuals of larger body size expire relatively less respiratory moisture than those of smaller size. They suggest that this phenomenon is associated in some way with the higher metabolic rates of the organisms of smaller size.

Since respiratory water loss (vaporization) increases as the surrounding temperature approaches that of the bird's body (and heat loss by radiation decreases; see the comments of Wallgren, 1954), heat dissipation in warm, humid climates is in all probability a critical problem in the adaptation of homeothermic organisms to this environment. If the relatively warm summer temperatures of the tropics increase the component of heat loss via vaporization, and if simultaneously the high humidities (viz., low saturation deficits of inspired air) tend to reduce the efficiency of heat loss via vaporization, then selection for decreased general body size might favor development and/or survival in warm, humid climates. Here, a relative increase in internal respiratory surfaces, along with slight increases in metabolic activities (e.g., respiratory rates?) might facilitate heat dissipation. This suggestion needs to be tested experimentally.

4. Interspecific Competition.—Various workers have discussed the role of size differences in bird species as a means of avoiding interspecific competition for limited, but critical resources of the environment (e.g., Lack, 1944, 1947; Rand, 1951), and Hutchinson and MacArthur (1959) have recently theorized on the relation between numbers of animal species and the size of component species.

The factor of competition is rarely considered in interpretation of ecogeographical variation in wing length and body size, probably because bird systematists have for the most part concentrated on geographic variation in species of temperate climates, and have neglected the study of tropical species (see, however, Moreau's study of the African *Zosterops* complex, 1957). Although little attention is directed to this topic in the present paper, competition should be considered as one possible factor contributing to the selection pressures that determine the body size of species that are members of rich tropical avifaunae. This might be particularly important for interpretation of the variation in wide-ranging species or superspecies that have populations both in temperate and tropical latitudes.

B. Selection Forces Directly Affecting the Structure of the Wing

1. Density of Vegetation.—In 1931, Miller suggested (in a study of populations of *Lanius ludovicianus* occurring in Sono-

ran Mexico) that individuals resident in regions of sparse or open vegetation may be characterized by relatively longer wings than conspecific individuals occurring elsewhere in denser or more closed stands of vegetation (see also Linsdale, 1938). Pitelka (1951) has reached similar conclusions for populations of *Aphelocoma coerulescens* in southern California.

One interpretation of this proposed ecogeographical rule is that a relatively shorter wing facilitates flight in or about dense vegetation, and therefore, in regions of open vegetation, a longer wing may be favored. Another interpretation is that in carrying out normal biological activities in regions of sparse or thin vegetation, birds have to fly greater distances than in regions of closed vegetation. Here it is assumed that a longer wing is an adaptation for increased flight activities in regions of open vegetation.

These explanations are undoubtedly far too simple for variation that results from morphological adjustments of the bird to its habitat by the agency of natural selection. For example, it is obvious for aerial species that the amount of time per day spent in foraging flight will be a factor determining the degree to which the structure of the vegetation influences length of wing, and different problems may be confronted by ground-foraging or arboreal species occurring in habitats of different densities. The plausibility of a theory such as that of Miller is, however, evident from intergeneric comparisons between the woodland species of the genus *Accipter* (which catch prey on the wing within vegetation, and have relatively short and blunted wings) and the falcons of the genus *Falco* (more aerial forms that catch prey on the wing in open areas, and have relatively longer and more pointed wings). The degree to which such adaptive modifications are manifested at the intraspecific level is a problem that remains to be analyzed. It should also be noted that exploitation of dense vegetation may result in reduced body size as well as in reduced length of wing.

2. *Pressure of Air.*—That wing length tends to become greater at higher altitudes for intraspecific comparisons was established early and is now well documented (Allen, 1871; Chapman and Griscom, 1924; Rand, 1936; Huxley, 1942; Mayr, 1942; Traylor, 1950). Comparative studies, such as those of Rand and Traylor on the New Guinean and Andean avifaunae, indicate the prevalence of this tendency. In the studies cited above, it was generally assumed that increased wing length at higher elevations is an indication of increased body size—viz., the operation of Bergmann's rule because of lower environmental temperatures at higher elevations. However, Stresemann (1941) raised the possibility that the adaptation for flight in the thin air of higher altitudes might necessitate a relatively larger surface and length of wing.

Moreau (1957, 1960) has conducted a partial regression analysis of the variation in wing length in the *Zosterops* complex of Africa. His study attempts to dissociate the effects of altitude (presumably being associated with vertical changes in air pressure) from the effects of environmental factors such as winter or summer temperature, and reveals a correlation between wing length and altitude that is independent of temperature. Moreau's discovery appears to be good evidence for the specific operation of a selection force immediately on the structure of the wing.

3. *Migratory Flight.*—Conclusions on the influence of migratory flight on intraspecific differences in length and structure of the bird wing are not yet definite. Numerous workers (Averill, 1925; Kipp, 1936; Meise, 1938; Rensch, 1934) have presented evidence that intraspecific adjustments in the structure of the wing—necessary for migrational flight—are effected via alterations in both the shape and length of the wing. In species containing both migratory and resident populations, these workers have noted that migratory individuals often have (i) more pointed wing-tips and (ii) greater length of wing

(see Mayr, 1942). They have also demonstrated (particularly Rensch) that a more pointed wing-tip is achieved by appropriate increases and decreases in the lengths of the primary feathers that compose the wing-tip. Thus a longer and/or more pointed wing seems to be necessary for coping with the demands of migrational flight. It is commonly assumed by ornithologists that an increase in wing length and area is necessary for greater periods of sustained flight, and that a pointed wing-tip facilitates rapid and long-distance flight. The degree of validity of these ideas has not been established.

Mayr and Vaurie (1948) have warned that migratory populations of a species may have wing lengths modified in comparison to resident populations, which, in fact, may obscure or override Bergmann's rule. Relating to this problem is Salomonsen's study on the evolutionary significance of migration. In developing one part of Salomonsen's thesis, I would like to use a historical approach—an approach that may be of interest to physiologists not familiar with the history of the development of the concept of climatic rules.

Rensch's studies (1939b) of the Palearctic passerine bird fauna revealed that not all wide ranging species of birds demonstrate Bergmann's rule; about 16% failed to conform. Some were continental species consisting of both northerly-breeding migratory populations and more southerly resident populations. And, it was more or less assumed by bird systematists that, because of the migrations of northern populations, these species were not subjected to the selective action of low winter temperatures. However, when distributions of populations during the breeding season were considered, a few of these species showed clinal increases in wing length toward the south—seemingly a reversal of Bergmann's rule.

In 1955, Salomonsen reported on "leap-frog" migration within many of the bird species that had previously been designated as reversals of Bergmann's rule. His findings, based both on banding returns and specimens, suggest (see, e.g., fig. 2)—when these migratory species are studied for intraspecific comparisons between wing length and temperature of the off-season, wintering quarters—that Bergmann's rule may still be manifested. In brief, he finds for numerous Palearctic bird species that northern-breeding populations may tend to winter further south (hence, "leap-frog" migration) than their southern-breeding, conspecific counterparts. Thus, the resident breeding populations of the middle or temperate latitudes (over which the conspecific populations breeding in the north migrate) are exposed to colder winter temperatures.

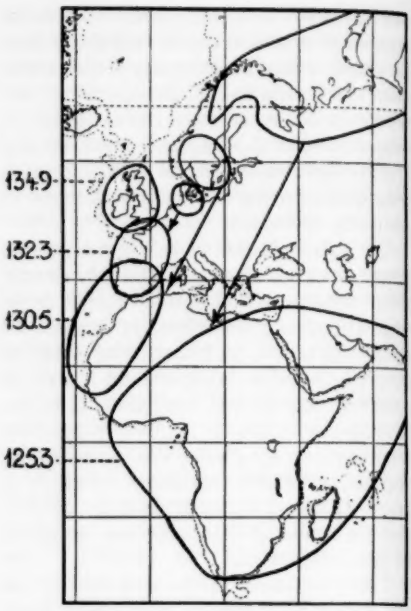

FIG. 2. Relation between wing length, migration, and geographic location of wintering areas in a "leap-frog" migratory species of plover (*Charadrius hiaticula*). Breeding areas and estimated winter-quarters are based on collected specimens and on recoveries of banded birds. Reproduced with the author's permission from Salomonsen, 1955.

The fact that resident populations of Palearctic species restricted to climates of

middle latitudes (e.g., central Europe) tend to have greater wing lengths and body sizes than their northern migratory counterparts is evidence for the operation of cold temperature on general body size. It illustrates the importance of considering the climate of the winter quarters in studies of ecogeographical variation in species containing migratory as well as resident populations. Even though these migratory populations of the north are not subjected to severe winter temperatures, other factors may influence the length of their wings. For example, in figure 2, the variation in *Charadrius hiaticula* reveals that northern-breeding populations, strongly migratory, have relatively small wing lengths. This may indicate: (i) the operation of Bergmann's rule on local populations resident in England or western Europe because of their restriction to regions of cold temperatures during the winter of the northern hemisphere and/or (ii) a reduction in general body size necessary for populations migrating long distances. Suggestion (ii) is here presented as an additional, and not as an alternative, explanation to Salomonsen's suggestion (i) for the adaptive significance of the variation in *C. hiaticula*.

Dr. Ernst Mayr has suggested (pers. comm.) that a lower mean body weight may require relatively less energy for migratory flight, and thereby may permit a quicker restoration of expended fat deposits. Cases of rapid deposition of fat during migratory stopovers may have a bearing on this possibility (see Odum and Perkinson, 1951).

Is body weight a valid indicator of body size in birds?—If wing length is not always the best indication of body size, what character should be used in the study of geographic variation in size? Body weight is sometimes used, but even this measurement is suspect, for the weights of some migratory individuals may fluctuate more than twenty percent during a twenty-four hour period. There is also the problem of what is meant by "size." For example, is the northern increase in body weight of the sedentary populations of *Richmondena cardinalis* (cited above) truly indicative of a size increase? Dr. Carl Helms has suggested (pers. comm.) that for this species the increase in body weight might be due to greater levels of deposited fat (as an auxiliary adaptation for winter survival), and not to an over-all increase in body size. Connell et al. (1960) have recently demonstrated that fat-free body weights parallel wing length, and thus offer a good measure of body size. These workers studied migratory individuals and found, for *Vireo olivaceus*, that a migrating male may weigh from 16 to 28 grams, while its corrected, fat-free weight will vary from 15 by only a gram. Their data suggest that wing length may be a valid indicator of fat-free body size in some species.

These comments on the interrelations of wing length, migration, weight, and size are inconclusive, and are presented here only as an outline of the numerous problems that remain to be examined. What is needed is a study of variation in fat deposition, fat-free body weight, wing length, and shape of wing-tip in a species (such as the plover species of figure 2) having both migratory and resident populations. Approaching this is the study by Dilger (1956), which reveals for the thrush genus *Catharus* that the most aerial and migratory species has a lower body weight and a relatively longer wing than its congeners which are less aerial and migratory. His study indicates that the humerus is a fair indicator of size (see table 1).

Discussion

1. Concomitant Operation of Ecogeographical Rules.—Comparing the ecogeographic trends for intraspecific variations in wing length among various bird species, many of which are resident to different continental climatic regions, the following concomitant operations seem evident for some arbitrarily chosen environments:

environment	*stringent climatic season*	*possible selection factor (s)*
Higher continental latitudes		
	winter	brief feeding day
a. resident species	winter	low temperatures
b. migratory species	that of winter-quarters?	—— ? ——
	———	migratory flight
Middle continental latitudes		
	winter	low temperatures
a. resident species	winter	brief feeding day
b. migratory species	that of winter-quarters?	—— ? ——
	———	migratory flight
Arid, subtropical lowlands		
	winter	low temperatures
(mostly resident species)	———	open vegetation
	summer	aridity
Mesic, subtropical mountains		
	winter	low temperatures
(mostly resident species)	———	reduced air pressure
	summer	aridity
Humid, tropical lowlands		
(mostly resident species)	summer	high humidities and temperatures
	———	dense vegetation
	———	interspecific competition
Humid, tropical mountains		
	winter	low temperatures
(mostly resident species)	———	reduced air pressure

From the outline above, it seems safe to postulate that ecogeographic variation at the intraspecific level is generally achieved by the operation of more than one selection force.

2. *Relative Importance of the Rules.*—The conclusion of combined operation of ecogeographical rules is not, unfortunately, a blessing in disguise to the evolutionist. If more than one selection force provides an explanation, then how are we to determine causality with confidence in evaluating the ecogeographical rules? For example, take the populations of *Parus major* restricted to the mountains of North Africa (fig. 1). Are their relatively greater wing lengths due to cold temperatures in the winter, to the reduced air pressure, or to the summer aridity of the region? On the other hand, could the increase in wing length be a result of all three factors contributing to an additive selection pressure at a latitude in which the relatively long winter feeding day does not suppress size increases? With the tools systematists now have (excluding, perhaps, partial regression analysis), it seems rather futile to attempt to resolve this problem on a quantitative basis.

3. *The Nature of Climatic Adaptation in Birds and Mammals.*—If one surveys—at the intraspecific and interspecific level—the adaptations of mammals to different climates, a variety of adaptations seems available for achieving approximately the same biologic function (i.e., appropriate heat economy). For effective heat dissipation in the tropics, the following adaptations have been recognized either by physiologists or evolutionists: (i) decrease in effectiveness of external insulation; (ii) lowering of metabolic activities; and (iii) reduction of general body size. This listing is surely an oversimplification.

Data on the occurrence and functions of the first trend are numerous (Scholander, 1955). The second trend is problematic, and is cited here as a possible explanation for the strange case of the sloth which, although having a thick and heavy external insulation of fur, occurs in the tropical rain forest. The low metabolism of this

TABLE 1. *Species differences in body weight and wing length in relation to some bone measurements for five species of the thrush genus* Catharus *(data from Dilger, 1956)*

Ground-dwelling and -foraging	→				Increasing arboreal foraging and aerial activity
species characteristic	*occidentalis*	*minimus*	*Catharus:*[1] *guttatus*	*fuscescens*	*ustulatus*
Body weight:	27.0 g	28.4	31.0	32.5	29.2
Wing length:	88.8 mm	92.2	93.2	98.2	98.8
Length of wing elements:					
humerus:	19.9	19.9	20.6	21.2	20.4
ulna:	23.6	24.2	26.6	27.0	26.1
manus:	19.8	22.1	23.0	24.4	23.9
Length of leg elements:					
femur:	20.5	20.7	20.4	21.3	20.3
tarsus:	33.5	28.9	29.8	29.4	27.1
Length of migration route:[2]	sedentary (Mexico)	1000–2500 miles	100–1500 miles	2000–4000 miles	3000–5000 miles
Most distant wintering area:	——	northern South America	southern United States	central South America	southern South America

[1] Excepting the Mexican species, *C. occidentalis*, these are North American, and are represented here by measurements of populations (sampled by Dilger) from New York State. *Catharus ustulatus*, the most migratory and aerial of these five species, is of interest since it appears to have a lengthened wing (for its size or body weight) or (?) a reduced general body size (witness decreases in bone measurements). See Dilger (1956) for the statistics of the mean values.

[2] An approximation based on a variety of sources.

sluggish species may be associated in some way with the presence of a heavy external insulation (regardless of its function), and could serve in part to prevent excessive heating of the body during the seasons or daily hours of high temperatures and humidities. This, of course, implies that the sloths are now relicts and that their ancestors were adapted to other, probably temperate, environments. The validity of the third adaptive trend also has yet to be adequately documented. Both Simpson (1953a) and Hesse et al. (1951) have observed that homeothermic animals tend to be smaller in hot climatic regions. If this trend of intraspecific adaptation really occurs in mammals, its multiple causes may well be those that seem to be responsible for the same phenomenon in birds: effective heat dissipation, competition, or dense vegetation.

If natural selection in mammals has operated for climatic adaptation along alternative pathways, might not the same be true for birds at both the intraspecific and interspecific levels? Combining the data of Steen (1958) and Snow (1954), it is apparent that these workers—independently and respectively studying the physiological and morphological adaptations of Scandinavian individuals of *Parus major*—have demonstrated manifestations of each of the three adaptive devices cited above for climatic adaptation in mammals. Individuals of *P. major*, Steen finds, survive the low temperatures of the arctic night by seeking cover, by huddling in groups, by balling-up the feathers (which increases external insulation), and by lowering the body temperature (hypothermia). Snow's ecogeographic analysis suggests that these Scandinavian individuals have slightly reduced body sizes, apparently correlated with the short feeding day of winter (op.

cit.). Here, both nocturnal hypothermia and slight reductions in over-all body size might interact to facilitate alleviation of the problem of restricted time for food intake during the arctic winter.

Thus, for climatic adaptation both in mammals and in birds, the same basic physiomorphological devices seem available. The major differences seem to lie in the combinations that are utilized by selection in different groups of organisms, or in the degree to which one adaptive device is emphasized or strengthened over another. From this viewpoint, it is not difficult to reconcile the differences between the adaptation physiologists and population evolutionists alluded to at the beginning of this paper. Basically both groups are dealing with variants of the same phenomenon—climatic adaptation involving multiple adaptive responses.

4. The Adaptive Meanings of the Ecogeographic Rules.—An ecogeographical rule is nothing more than an empirical observation associating change in character of conspecific populations with that of an environmental factor (i.e., Huxley's definition of a cline). The nature and the mechanism of the selection forces that elicit such adaptive responses are of course the unknowns about which biologists have long hypothesized. The preceding portions of this paper represent a comparison of the *apparent* actions and interactions of seven or eight selection forces postulated as exerting intraspecific influences on the wing or body size of the bird. We often ask, what are the adaptive meanings of these rules? The answers are yet to come because we lack both the data and the techniques for testing the hypotheses we put forward. The study of adaptation demands careful distinction between cause and function, but the literature for ecogeographical variation is resplendent with causal theories for adaptations whose survival or "fitness" values are completely unknown.

5. Bergmann's Rule as an Evolutionary Problem.—It is becoming increasingly evident that adaptation to the environment is far more complex than previously thought. Distinctions between "climatic," "physiological," "ecological," "ecogeographical," and "reproductive" adaptations to, say, the arctic environment are real only insofar as a researcher sets boundaries to his investigation. All adaptations are compound, and one might say that an organism is a mosaic of adapted characters—each of which serves for one or more functions, and each of which is co-adapted to the functions of others. For some time biologists have maintained that there is a heat-conserving principle underlying Bergmann's rule. In 1955, Scholander denied this, and suggested that for homeotherms heat dissipation is the "only major phylogenetic adaptation to climate." Suggesting for intraspecific comparisons that (i) basal heat production "has been found to be essentially the same in all climates" and that (ii) heat dissipation is achieved mostly by adaptations associated with changes in external insulation and temperature-tolerances of tissues, he concludes for homeotherms that "there is no physiological evidence . . . [that] . . . Bergmann's and Allen's rules reflect phylogenetic pathways of heat-conserving adaptation." For evaluation of the adaptive significance of Bergmann's rule, is this the most adequate conclusion for the data available from the literature of comparative physiology? The following five paragraphs refer to this matter:

a. Heat dissipation in homeotherms may be realized by a variety of channels: radiation, vaporization, conduction, and convection (Salt, 1952). It is logical to expect that the relative importance of these channels may vary between nonconspecific organisms, as well as between conspecific individuals, occurring in different seasonal or geographic environments. Furthermore, heat regulation is also a problem at various stages of development for the homeothermic organism, and various factors of the environment are expected to exert selective influences on the newly born or hatched, as well as on the adult. When

such possibilities are considered, it becomes clear that Scholander's data pertain primarily to problems associated with heat dissipation by radiation in adult homeotherms. How much one can conclude on the problem of adaptation from the study of adult acclimation is a matter of considerable interest (e.g., compare Scholander's views with those of Hart, 1957). It does seem evident, however, that more information is needed on (i) the stage(s) of development at which various climatic selection pressures operate on individuals of natural populations and (ii) on the relative importance of the different channels for heat dissipation in different environments. For example, Wallgren (1954) has demonstrated for two species of *Emberiza* occurring in Europe that at 30° C as much as 40 per cent of the total heat loss is dissipated via vaporization.

b. My comments (cited above) on the methodology whereby the adaptation physiologists study homeothermic acclimation and adaptation ignore a vast literature dealing with studies of heat regulation, tissue tolerance, peripheral circulation, nerve control, behavior, and other devices, processes, or activities for achievement of thermoregulation. For this information, and for a discussion of the differences between temperature-induced changes and climate-induced changes in birds and mammals, see the review of Hart (1957). This comparative physiologist concludes (p. 167) his review with the following observation: "Physiological adjustments to natural climatic conditions can be expected to be more varied than acclimation because of the greater diversity in environmental experience. . . . The response to climate is thus viewed as a resultant of multiple effects of various environmental factors in nature in contrast to acclimation resulting from the action of a single known factor."

c. The adaptive significance (*sensu stricto*) of Bergmann's rule is to be found in whether or not slight increases in size or bulk, with concomitant decreases in relative surface area, facilitate development, survival, or achievement of heat economy at low environmental temperatures. Biologists have long thought about this problem, and numerous researchers have noted (see, e.g., Sumner, 1909, 1915; Sundstroem, 1944; Przibram, 1925; Allee and Lutherman, 1940), that rodents and domestic chickens raised at higher and more humid temperatures tend to be smaller in general body size and to have relatively larger appendages than individuals raised at lower temperatures. No one has demonstrated that these experiments extended over a period of generations will result in genotypic as well as phenotypic shifts (i.e., the Baldwin effect *sensu* Simpson, 1953a), but this is one possible way (see Ray, 1960) the process of climatic adaptation might occur in nature.

d. Harrison (1958) has recently reconfirmed the early experiments of Sumner, in addition to examining the effects of tail amputation on the heat tolerance of mice exposed to lethal temperatures. He finds that mice whose tails have been amputated (two weeks before testing) have a lower heat tolerance for survival than mice possessing tails. He concludes (for phenotypic adjustments only) that "at least some of the changes, both physiological and morphological, which occur when mice are raised at high temperatures, are in their over-all effect adaptive." From such data, I conclude that slight reductions in general body size partly facilitate achievement of effective heat dissipation for the homeotherm under warm and humid conditions. Reciprocally, slight increases in size are expected to promote achievement of heat conservation under cold conditions.

e. For a more valid analysis of Bergmann's rule, a study is needed of the survival values or cold tolerances of conspecific individuals of different genetic sizes when exposed to lethal or nonlethal, low temperatures. However, since we have experimental evidence that a reduced size for some homeothermic organisms is more favorable for development at high temperatures, the commonly accepted theory

of a physiological basis for Bergmann's rule may be considered valid until comparable evidence is brought forward to show that a larger size is not more advantageous than a smaller size for development (or survival) at relatively low temperatures.

6. Phylogenetic Results of Ecogeographic Variation.—I am grateful to Dr. Ernst Mayr for pointing out to me the possible usefulness (since there are so many selective advantages for a homeotherm with increased body size) of relating the ecogeographical data presented above to the following evolutionary theory. Although evolution may not always proceed for increases in the size of the individual,[3] many fossil series, both vertebrate and invertebrate, exhibit phyletic trends for increase in bulk or body size (see Rensch, 1948, 1960; Newell, 1949; Simpson, 1953b). Since most neo-darwinians now believe that phyletic evolution is essentially no more than adaptation resulting from microevolutionary changes (i.e., the results of the action of natural selection on the genetic variation realized in local populations), it might justifiably be argued for homeotherms that (i) these trends for size changes are manifested, in varying degrees, by contemporary populations and species, and that (ii) the factors responsible for the ecogeographical rules are also those that provide the initial sources of the changes associated with phyletic size evolution. Operating on these changes, factors such as isolation, degree of inter-isolate gene-exchange, and competition may subsequently lead to different ecologic adjustments, and thus eventually obscure ecogeographic trends of variation. Theoretically, the ecographic trends are expected to be modified and obscured when certain selection pressures begin to operate specifically for adaptive changes in the ecological niche-habitat requirements of populations or species (see Snow, 1954).

It is contended, therefore, that outside the humid, tropical lowlands, at least one selection force—viz., warm humidity—on bird populations for decreased body size is relaxed, thereby permitting a variety of other selection forces to operate for increases in body size—e.g., in the colder latitudes, at higher elevations, or in warm, arid regions. Is this a valid conclusion, and, if so, are similar comments valid for mammals (see, however, Darlington, 1959, p. 507)?

This new "view" of ecogeographic variation in birds in no way contradicts the previous conclusions of this paper, and has the conceptual advantage of again suggesting that a variety of selection forces and a variety of adaptive responses are involved in the process of climatic adaptation in birds.[4] It is hoped that this will encourage others to investigate what may prove to be a challenging area of future research—that of the adaptive interactions between populations and their biotic environments, when studied from the viewpoint of alternate evolutionary pathways.

Conclusions

1. Intraspecific trends of variation in wing length and body size in birds are real and exist in correlation with various gradient factors of the environment. Although each may show clinal variation, wing length and body size do not always vary concordantly or concomitantly. The degree of correlation (primary or secondary) between wing length and body size and most environmental factors is uncertain.

2. For most cases of ecogeographical variation in wing length and body size, more than one selection force is apparently in operation. The adaptive significance of these trends or rules is hypothetical, with the possible exception of Bergmann's rule. The adaptive meaning of this rule seems

[3] Simpson (1953b, pp. 151–152) enumerates some special circumstances which favor small size in animals: "very warm climate for homothermous animals, insular conditions, subterranean life, limitation of food supply, greater opportunities for escape from enemies."

[4] I have not attempted to relate these ideas to Darlington's (1959) and Brown's (1959) concepts of general and special adaptations.

evident from experimentation, but even this remains to be "proven."

3. The physiomorphological devices utilized for homeothermic climatic adaptation are basically the same in mammals and birds. The differences lie in the combinations of heat-regulating devices and/or in the degree to which one device is (or several are) favored or enhanced over others by selection. This refers more to the problem of evolutionary adaptation at the population and species level than to the problem of individual adaptability at the level of the organism. The two problems, however, are interrelated, and, for Bergmann's rule, the Baldwin effect is considered to be the mechanism whereby initial phenotypic adjustments come under genetic control.

4. It is suggested for intraspecific variation in size among some bird species that the environment of warm and humid continental regions in some way tends to suppress increases in general body size. This may relate to developmental or survival problems associated with effective heat dissipation. Here, however, some other selection forces (dense vegetation, interspecific competition) might also operate for slight reductions in size at the population or species level.

5. The above theory is interpreted to mean that, during the process of speciation and geographic spread of populations and species, birds meeting and exploiting new environments tend to become larger. This is not to deny that natural selection (in time and/or in special circumstances) will elicit new, more favorable combinations of adaptive responses, and thereby operate, depending on specific ecological demands, for larger or smaller body sizes in tropical as well as non-tropical regions.

6. Examining both birds and mammals for adaptive interactions between populations and their environments, natural selection for climatic adaptation seems to have (a) adjusted the efficiency of external insulation, and/or (b) regulated metabolic activities controlling heat production and dissipation, and/or (c) altered size and surface area ratios, and/or (of course) modified other physiomorphological devices or behavior traits which also effect thermoregulation. This theory of multiple evolutionary pathways for achievement of heat economy raises the possibility that the conceptual schism between the physiologists and the evolutionists on the topic of the adaptive meanings of the climatic rules is more apparent than real.

Acknowledgments

I am greatly indebted to Dr. Ernst Mayr for criticism and helpful suggestions throughout this study. Special recognition is also due to Dr. A. L. Rand for criticizing the concepts developed in the present paper. Dr. D. R. Griffin kindly reminded me that the problem of the physiological meaning of Bergmann's rule will be resolved only when we have adequate experimental data on changes in heat conservation and dissipation with slight changes in body size of homeothermic individuals. The recent experiments and review of Harrison (1958) and Hart (1957) are relevant to this, and deserve more attention. Finally, the following have also kindly read the manuscript and/or made helpful suggestions: R. E. Moreau, W. L. Brown, Jr., P. J. Darlington, F. A. Pitelka, K. L. Dixon, A. S. Rand, G. Bush, W. J. Bock, W. John Smith, C. W. Helms, R. H. Barth, Jr., and R. W. Thorington, Jr.

Epilogue

Since the above was written, I have seen the manuscript of Dr. R. A. Paynter, Jr., which deals with the variation in the Indo-Pakistan segments of *Corvus macrorhynchus*. His data indicate for these populations a direct relation between altitude and wing length, but an inverse relation between altitude and body weight. This finding—which suggests that intraspecific reduction in body weight may be another adaptation (see Stresemann, 1939) facilitating alleviation of problems associated with flight and/or (?) feeding ecology at

higher elevations—demonstrates again that different selection forces may be in operation on both the body and the wing of the bird.

Incidentally, it should be noted that Rensch (1960, p. 46) cites the papers of Winkel (1951; *Zool. Jb., Abt. Syst., 80*: 256–276) and Saxena (1957; *Zeit. f. vergl. Physiol., 40*: 367–396) as contradicting Scholander's argument that there is no physiological basis to Bergmann's rule. I am not familiar with the latter two papers.

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BLOOD GROUPS AND THE TAXONOMIC STATUS OF AMERICAN BUFFALO AND DOMESTIC CATTLE

C. Stormont, W. J. Miller, and Y. Suzuki
School of Veterinary Medicine, University of California, Davis, California

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The generally accepted taxonomic position of the American buffalo (*Bison bison*) and domestic cattle (specifically, *Bos taurus*) is that of separate genera in the subfamily Bovinae. That the two species might very well occupy positions in the same genus is suggested by one of several alternative taxonomic designations for the American buffalo, notably, *Bos americanus* (see Roe, 1951). The two species are apparently fully cross-fertile, but only the females among the F_1 hybrids seem to be fertile (Deakin et al., 1942). Attempts to differentiate the two species by means of the classical precipitin technique have not been uniformly successful (Wolfe, 1939) and even when turbidimetric methods of measuring precipitates are used, the two species always stand closely side by side (Boyden et al., 1951). In this connection the question arises whether more extreme differences in precipitin values might be found between and perhaps even within breeds of domestic cattle. Likewise, attempts to demonstrate a clearly definitive difference between the two species by means of cross-absorbing antisera produced in rabbits against both bison and cattle red cells yielded equivocal results (Owen et al., 1958). In the case of each antiserum, absorption with a pool of red cells from the heterologous species succeeded in removing the greater portion and sometimes all of the antibodies directed against the red cells of the homologous species. Such observations suggest that the antigenic structure of the red cells of bison and domestic cattle must be very similar, and this was borne out to a considerable degree by blood typing bison with a rather large battery of blood-typing antibodies (reagents) which had been developed specifically for the purpose of exploring individual differences in cattle. For example, Owen et al. observed antigenic factors in bison blood which appeared to be indistinguishable from the bovine blood factors named **A**, **C**, **J**, **S**, **W**, **X**, and **Z**. Nevertheless, some rather marked differences were noted between the blood types of the two species, and those differences appeared to be significantly greater than any of the between-breed differences encountered in blood typing domestic cattle. For example, it appeared that the complex B locus of cattle is either not represented in bison or, if present, is occupied solely by alleles whose products (phenogroups) are essentially non-reactive with any of the 21 B system reagents used in that study.

The data reported by Owen et al. (1958) were actually collected during the years 1945–47. Since then, we have had the opportunity to develop in this laboratory an entirely new battery of cattle blood-typing reagents which derive from antisera independent of those used as sources of reagents by Owen et al. or by Miller and Stone (1955) in a further study of bison blood types. In connection with another study (Stormont and Suzuki, 1958), we had the opportunity to type three bison with our reagents. The results of those tests provided evidence which tended to narrow the magnitude of the blood-type differences between the two species. Further inquiry was therefore prompted and through the courtesy of Mr. Julian A. Howard, Refuge Manager of the Wichita Mountains Wild Life Refuge, Cache, Oklahoma, and Dr. D. C. Bostwick, we received 56 samples of citrated bison blood on October 29, 1959. (This was the same herd from which Owen et al. obtained 44 of the 69 samples used in their study, and it was also the same herd from which

TABLE 1. *The 41 classified "facsimile" reagents used in typing the 56 bison blood samples*[1]
(Information is given on the number of replicas of each reagent, as shown within parentheses; the letters a, b, c, ... etc. ... m indicate the sources of the antisera from which the reagents were obtained, and the decode for these letters is given as follows: a = cattle isoimmune serum; b = rabbit anti-cattle serum; c = sheep isoimmune serum; d = rabbit anti-bison serum; e = rabbit anti-bactrian camel serum; f = goat anti-cattle serum; g = sheep anti-cattle serum; h = rabbit anti-human A serum; i = cattle anti-human A serum; j = normal cattle serum; k = normal sheep serum; l = normal pronghorn antelope serum; m = cattle anti-bison serum.)

Reagents by systems	Sources and replicas	Reagents by systems	Sources and replicas	Reagents by systems	Sources and replicas
A system		*B system*		*F–V system*	
A_1	a (1)	A′	a (1)	F	b (2); d (1); e (1); f (1); k (1); l (2)
A_2	b (1)	D′	a (1)	V	a (3); b (1); g (1); m (1)
H	a (2)	E_1′	a (1); b (1)	*J system*	
B system		E_2′	a (1)	J	h (3); i (1); j (2)
B	a (1)	E_3′	a (1); c (1)	*L system*	
G	a (5)	I′	a (2)	L	a (1)
I	a (1); c (1)	J′	a (2)	*S system*	
K	a (2)	K′	a (3)	S	a (2)
O_1	a (1)	*C system*		U_1	a (1); b (1)
O_2	a (1)	C_1	a (2)	U_2	a (1)
P	a (1)	C_2	a (1)	U′	a (1)
Q	a (1)	R	a (3); c (1)	H′	a (2)
T_1	a (1)	W	a (2)	*Z system*	
T_2	a (1)	X_1	a (1); d (1)	Z	a (2); b (1)
Y_1	a (1)	X_2	a (1)	*Z′ system*	
Y_2	a (2)	L′	a (6)	Z′	a (1)

[1] Facsimile reagents are simply reagents which in blood typing cattle are known to be replicas or near-replicas of cattle reagents which have been previously described in the literature.

Miller and Stone obtained 193 samples for their study.) The present report is concerned with the results of blood typing those 56 samples. Data are also presented on the occurrence of certain natural antibodies and soluble blood group substances in the plasma of the bison blood samples.

MATERIALS AND METHODS

Hemolytic techniques, as described in previous reports cited by Owen et al. (1958), were used in this as well as the two previous studies. The only deviation in technique of the present study concerned sources of complement. Heretofore, fresh frozen rabbit sera has been used exclusively as complement in cattle and bison blood typing. We have observed, however, that a few of our reagents, particularly our anti-J reagents prepared from rabbit antisera engendered by immunizing with human red cells of type A (Stormont and Suzuki, 1960), have little or no lytic activity when complemented with fresh rabbit sera but do promote specific hemolysis when activated by fresh guinea pig sera used diluted in a range of 1:15 to 1:20.

In table 1 we have listed 41 cattle blood-typing reagents used in the present study which are known to be facsimiles or near-facsimiles in tests of cattle of the vast majority of the reagents used in the two previous studies. We also have noted in table 1 the source of each antiserum from which the various reagents were derived and the number of replicas of each reagent.

In table 2 are listed the new reagents, a total of 25, whose reactions with bison red cells have not previously been described. As in table 1, we have indicated in table 2 the sources of antisera from which the new reagents were derived and also the number of replicas of each reagent.

TABLE 2. *The 25 classified "new" reagents used in typing the 56 bison bloods*

(The number of replicas of each reagent is shown within parentheses. The decode of the sources of antisera is as follows: a = cattle isoimmune serum; b = rabbit anti-cattle serum; c = sheep isoimmune serum; d = normal cattle serum.)

Reagents by systems	Sources and replicas
A system	
D	b (7)
B system	
B_2	a (1)
I_3	a (2)
O_x	a (1)
P_x	a (1)
A_3'	a (1)
B′ or NF6	a (2)
E_x'	a (4)
F′	a (1)
G′	a (2)
O′ or NF8	a (1)
Y′ or NF4	a (1)
NF7	a (1)
S2	c (1)
S5	c (1)
S26	c (1)
S27	c (1)
S34	c (1)
C system	
E	a (2)
NF12	a (1)
J system	
Oc	d (1)
M system	
M_1	a (1)
M_2	a (1)
S system	
S + H′	a (2)
U_1 + H′	a (1); b(1)

All the reagents listed in tables 1 and 2 are classified reagents. That is, they have been classified with respect to the known genetic systems of blood groups in cattle in which they react (see Stormont, 1958). Attention is called to the reclassification of H′ reagent, which in the two previous studies had been regarded as being reactive in a system (the H′ system) independent of the 10 systems listed in tables 1 and 2. We have shown that H′ reagent reacts in the S system of bovine blood groups. Likewise, attention is called to a reclassification of the new D reagent (table 2) which was previously believed (Stormont and Suzuki, 1956) to be reactive in a new system (the D system) independent of the others. As pointed out in a brief note published elsewhere (Stormont et al., 1960), it was the results of typing the present sample of 56 bison bloods which provided the first clue (see table 3) relating the reactions of D antibodies to the A system of cattle.

RESULTS

Bona fide or true cross reactions, as determined by appropriate absorptions, were obtained in typing bison red cells with the facsimile reagents A_1, A_2, C_1, C_2, F, J, L, S, V, W, X_1, X_2, Z, and E_3' of table 1 and also with the new reagents D, M_2, P_x, S + H′, one of the two U_1 + H′, E_x' and NF (new factor) 12 of table 2. All the remaining reagents (27 in table 1 and 18 in table 2) produced no bona fide cross reactions with the red cells of bison. In this connection, however, we should mention that one of the five replicated G reagents (table 1) produced lysis of all 56 bison bloods but that the reactions, as readily demonstrated by absorbing the particular reagent with both bison red cells and with G-positive cattle cells, could not be attributed to the specific G antibodies in the reagent. Rather, they were due to certain heterophile antibodies which were probably present in the normal serum of the cow which produced the antiserum. At any rate, absorption of this particular G reagent with G-positive cattle red cells provided us with a testing fluid which could be used to distinguish bison red cells from those obtained from domestic cattle.

Summarized in table 3 are the reactions of the 15 reagents which distinguished individual differences in the 56 bison bloods. By means of appropriate symbols (see table 3), we have attempted to differentiate partial or incomplete cross reactions from complete cross reactions believed to be fully equivalent to the reactions obtained with the particular reagents in typing animals of the homologous species. Likewise, dosage reactions (indicative of two doses of a given blood factor) are differentiated

TABLE 3. *Summary of the reactions of 56 bison blood samples with 15 blood-typing reagents which disclosed individual differences*

(The dot [·] symbol indicates no hemolysis; the plus [+] symbol indicates hemolysis equivalent to that seen in typing cattle bloods; the plus–minus [±] symbol indicates a "partial" cross reaction in which hemolysis was not as intense as that regularly encountered in typing cattle; the letter d attached to the symbols ± and + indicates dosage reactions. In the J system, the symbol +1 indicates strong hemolysis with all J reagents; +2 indicates strong lysis with certain J reagents from rabbit anti-human A serum but somewhat weaker lysis with other J reagents; +3 indicates weaker lysis with strong J reagents and considerably weaker or even no hemolysis with other J reagents.)

Bison	Sex	Age	Reagents by Systems: A			B			C			J	M	S			Z
			A_1	A_2	D	P_x	E_2'	E_x'	C_1	C_2	W	J	M_2	S	S+H′	U_1+H′	Z
26	M	4	·	·	·	±	±	+	·	·	·	+1	·	+	+	±	+d
27	M	2	·	·	·	±	±	+	·	·	+	+1	·	+	+	±	·
28	M	2	·	·	·	·	·	·	·	·	·	+1	·	·	·	·	+d
29	M	4	·	·	·	·	·	·	·	·	+	+2	+	·	·	·	+
30	M	2	·	·	·	±	±	+	·	·	+	+1	+	·	·	·	+
31	M	2	·	·	·	·	·	·	·	·	+	+1	·	+	+	±d	+
32	M	2	+	+	±	·	·	·	·	·	·	+1	·	+	+	±	+
33	M	2	+	+	±	·	·	·	·	+	+	+1	·	·	·	·	·
34	M	3	+	+	±	±d	±d	+	·	·	·	+1	·	·	·	·	+d
35	M	4	+	+	±	±	±	+	·	·	·	·	·	·	·	·	+
36	M	2	·	·	·	·	·	·	·	+	+	+3	·	+	+	±	+
37	M	2	+	+	±	·	·	·	·	·	·	+1	·	·	·	·	+d
38	M	2	·	·	·	·	·	·	·	·	·	+1	·	·	·	·	+d
39	M	2	+	+	±	±	±	+	·	+	+	+1	·	·	·	·	+d
40	F	8	+	+	±	·	·	·	·	·	·	+2	·	·	·	·	+d
41	F	4	·	·	·	·	·	·	·	+	+	+1	·	+	+	±	·
42	F	4	·	·	·	·	·	·	·	·	+	+3	·	·	·	·	·
43	M	2	+	+	±	·	·	·	·	+	+	+1	·	+	+	±	·
44	M	10	+	+	±	±	±	+	·	+	+	+1	+	·	·	·	+
45	M	3	·	·	·	·	·	·	·	·	+	+1	·	+	+	±	·
46	M	3	+	+	±	·	·	·	·	·	·	·	·	·	·	·	+d
47	M	2	+	+	±	±	±	+	±d	+d	+	+1	·	+	+	±	+
48	M	3	+	+	±	·	·	·	·	·	·	+1	·	·	·	·	+d
49	M	2	·	·	·	±	±	+	·	·	·	+3	·	·	·	·	+d
50	M	2	·	·	·	±d	±d	+	·	·	·	+2	·	+	+	±	+
51*	?	?	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+
52	M	2	+	+	±	±d	±d	+	·	+	+	+1	·	·	·	·	+
53	M	2	+	+	±	±	±	+	·	·	·	+1	·	+	+	±	+d
54	M	2	·	·	·	±	±	+	·	·	·	+1	·	·	·	·	+d
55	M	2	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+d
56	M	2	·	·	·	±	±	+	·	·	·	+1	·	·	·	·	+
57	M	2	+	+	±	·	·	·	·	·	+	+1	·	+	+	±	+d
58	M	2	·	·	·	±	±	+	·	·	·	·	·	·	·	·	+
59*	?	?	+	+	±	·	·	·	·	·	+	+1	·	+	+	±d	+
60	F	3	·	·	·	·	·	·	·	·	+	·	·	·	·	·	+d
61*	?	?	+	+	±	±d	±d	+	·	·	·	+2	·	·	·	·	+
62	M	2	·	·	·	·	·	·	·	+	+	+2	·	·	·	·	+
63*	?	?	·	·	·	·	·	·	·	·	+	·	·	+	+	±	+
64	M	3	·	·	·	·	·	·	·	·	·	·	+	±	±	±	·
65	M	3	+	+	±	·	·	·	±d	+d	+	+2	·	+	+	±	+d
66	F	7	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+d
67	F	6	·	·	·	·	·	·	·	·	+	+1	·	·	·	·	·
68	F	3	+	+	±	±	±	+	·	·	+	+1	·	+	+	±	+
69	F	3	+	+	±	·	·	·	·	·	·	·	·	·	·	·	+d
70	F	6	·	·	·	·	·	·	·	·	+	+1	·	·	·	·	+
71	M	10	·	·	·	±	±	+	·	·	+	+1	+	·	·	·	+d
72	F	14	·	·	·	·	·	·	·	+	+	+1	·	+	+	±	+d
73	F	9	+	+	±	·	·	·	·	+	+	·	·	·	·	·	+d
74	F	3	·	·	·	·	·	·	·	·	+	·	·	·	·	·	+
75	F	4	·	·	·	·	·	·	·	·	+	+1	·	·	·	·	+
76	M	3	+	+	±	·	·	·	·	·	·	+1	·	·	·	·	+
77	M	3	·	·	·	±	±	+	·	·	+	+1	·	·	·	·	+d
78	F	3	·	·	·	±	±	+	·	·	+	+1	·	+	+	±	·
79	F	4	·	·	·	·	·	·	·	·	·	+1	·	+	+	±	+d
80	M	3	·	·	·	±	±	+	·	·	·	·	·	+	+	±	·
81	M	?	·	·	·	·	·	·	·	·	·	·	+	·	·	·	+

* Samples which lost their labels in transit. There were three males and one female.

and so, also, are the four main categories of J reactions. Note that the following pairs and trios had identical blood types: 28–38, 37–48, 46–69, 55–66–77, and 67–70–75.

The A System in Bison

Three of the four A system reagents used in typing cattle cross reacted with bison red cells, and all three distinguished individual differences. These were the reagents A_1, A_2, and D. However, each of these reagents produced the same reactions in the parallel tests, as shown in table 3. Consequently, a single pair of bison phenogroups A_1D and "–" (the negative alternative) is all that is required to explain

the reactions. The phenogroup A_1D cross reacted with A_1, A_2, and D antibodies whereas the "–" alternative was non-reactive with all of the A system reagents presently available. Estimates of the frequencies of the two alleles which control these phenogroups in the Oklahoma or Wichita population of bison are given in table 4.

The question arises whether the phenogroups A_1D and "–" of bison have their equivalents in domestic cattle. At the time these data were collected we could not be certain, since, as already noted, it was the present results which first called our attention to the fact that reagent D must be reacting not only in the A system of bison but also in the A system of cattle. Reanalysis of the A system of cattle has indicated (Stormont et al., 1960) that a minimum of seven phenogroups is required to explain the reactions of reagents A_1, A_2, D, and H with cattle bloods. Since all seven of these phenogroups exhibit positive reactions with one or more of the A system reagents, there is as yet no evidence for a phenogroup equivalent to the "–" alternative in bison. We have, however, observed an A system phenogroup in cattle which exhibits the same reactions as A_1D of bison. We have not had the opportunity to compare it with bison A_1D in absorptions with D reagent. As indicated in table 3, the D reactions of bison blood were not fully equivalent to those of cattle blood. When bison red cells of phenotype A_1D were used to absorb D reagents not all of the D antibodies for cattle were exhausted. However, in those tests there were no cattle which possessed phenogroup A_1D to the exclusion of other D-positive phenogroups. Further study is therefore required in order to decide whether A_1D of bison is equivalent to A_1D of cattle in the extent to which it will absorb D antibodies.

The B System in Bison

As indicated by the large number of B system reagents (tables 1 and 2) used in typing cattle, the B system of cattle is extremely complex. This is further indicated by the fact that we presently recognize, in our laboratory alone, 251 B phenogroups and their controlling alleles. A list of some of the B phenogroups of cattle and estimates of the frequencies of their alleles in several breeds of cattle are given elsewhere (Stormont, 1958).

Only three of the 37 B system reagents (tables 1 and 2) cross reacted with bison red cells. These were the reagents E_3' (facsimiles of the E_3' reagent used in two previous bison studies), P_x and E_x'. All three of these reagents are known to produce related patterns of cross reactions with cattle red cells. Each of the three reagents distinguished individual differences in bison, but, as shown in table 3, they produced the same reactions in the parallel tests.

As in the A system of bison, these results can be explained by postulating a single pair of bison phenogroups which we designate as E_x' and "–". Phenogroup E_x', as indicated by the results summarized in table 3, shows partial cross reactions with P_x and E_3' reagents but exhibits essentially complete cross reaction with the E_x' fraction of antibodies in the serologically complex E_x' subtyping reagents. As indicated in table 4, the "–" alternative in the bison B system has its homologue in domestic cattle, and that homologue occurs rather frequently in certain breeds such as Herefords and Shorthorns (see Stormont, 1958). There is no evidence so far for a B phenogroup in cattle which is equivalent to E_x' of bison. The closest resemblance to it is probably O_xE_3' of cattle. This phenogroup differs from E_x' of bison primarily by the fact that it cross reacts with O_x antibodies and also reacts more completely with E_3' and P_x antibodies than does phenogroup E_x' of bison. Estimates of the frequencies of the alleles which control phenogroups E_x' and "–" in the Oklahoma population of bison are given in table 4.

The C System in Bison

The C system is the second most complex system of blood groups recognized in cattle. Over 30 C phenogroups and their controlling alleles are presently known.

All the C system reagents (table 1 and table 2) except R, L′, and E produced bona-fide cross reactions with bison red cells. The two facsimile reagents X_1 and X_2, and the new reagent designated NF12 (table 2) cross reacted intensely with all 56 bison bloods. The cross reaction of NF12 antibodies with all 56 bison bloods was fully expected in view of the fact that NF12 reacts with all C phenogroups in cattle which are characterized by either or both blood factors **R** and **X**.

The reactions of the three C system reagents, notably C_1, C_2, and W, which distinguished individual differences in bison are summarized in table 3.

The present results with C system reagents, we believe, can be attributed to a minimum of three bison phenogroups designated X_1, WX_1, and C_2WX_1. A fourth C phenogroup, designated C_1WX_1, might be indicated by the weak or partial cross reactions obtained with our two C_1 reagents in tests of the red cells of bison numbered 47 and 65 in table 3. We have, however, for the purpose of the present report, taken the position that bison 47 and 65 were probably homozygous for phenogroup C_2WX_1 and that the weak reactions of their red cells with C_1 reagents resulted from a double dose of blood factor $\mathbf{C_2}$.

Estimates of the frequencies of the three alleles controlling phenogroups X_1, WX_1, and C_2WX_1 in the Oklahoma population of bison are given in table 4. Also, as noted in table 4, we have indicated that phenogroups X_1 and WX_1 have known homologues in domestic cattle but that, so far, there is no known homologue of C_2WX_1.

The F-V System in Bison

The F-V system in domestic cattle is typified by a single pair of phenogroups F and V which are controlled by a pair of alleles such that three phenotypes F (or F/F), FV (or F/V), and V (or V/V) are recognized. Recent observations in our laboratory suggest that the regular V phenogroup of cattle is divisible into two subgroups. Atypical variants of blood factor **V** have also been noted in this and other laboratories in typing *Bos indicus*, and there is suggestive evidence that variants of **F** also exist. In the present study, however, the reactions of bison red cells with F and V reagents were compared only with the regular F and V phenogroups of *Bos taurus*.

Partial cross reactions of all bison bloods of the present study were obtained with all of our F and V reagents (table 1) except in the case of the one F reagent obtained from a rabbit antiserum against the red cells of bison and the one V reagent obtained from a cattle antiserum against bison red cells. The reactions of the latter reagents were, of course, fully equivalent to those obtained with cattle red cells since, in these instances, bison rather than cattle represented the homologous species. However, two of the F reagents and three of the V reagents failed to produce visible hemolysis with bison red cells at the dilutions at which these reagents are ordinarily used in typing cattle. Nevertheless, absorption of these reagents as well as the other "partially" cross-reactive F and V reagents had a pronounced effect on the antibody populations. In each instance, the titer and degree of reactivity with cattle red cells were considerably reduced.

We propose that these results can be attributed to a single phenogroup in the F-V system of bison. We have designated this phenogroup as FV_b and note in table 4 that it does not presently have a known homologue in domestic cattle. Comparison of F-V variants in cattle with phenogroup FV_b of bison is a subject for further study.

That phenogroup FV_b of bison can be used to engender both F and V antibodies which will distinguish the three phenotypes F, FV, and V of domestic cattle is indicated by the sources of one of the F and one of the V reagents (table 1) used in the present study.

The J System in Bison

In the study of Owen et al., only one J reagent, a normal serum obtained from cow 17Z, was used. Nevertheless, it was possible, as in typing cattle, to distinguish

different degrees of reactivity with J-positive bison red cells. In the present study we employed three J reagents obtained by immunizing J-negative rabbits with human red cells of type A, one J reagent obtained by immunizing a J-negative cow with human red cells of type A, and two normal cattle serums which contained rather strong J isolysins (see table 1). With these reagents, as explained in the note to table 3 and as indicated by appropriate symbols in table 3, we were able to distinguish four main categories of J reactions which were typical of the four main categories of J reactions distinguished by these reagents in typing cattle bloods.

Although we propose that a minimum of four J alleles, with homologues in cattle, are required to explain these four categories, we made no attempt to distinguish between these alleles in estimating the gene frequencies given in table 4.

The blood plasmas of each of the 56 bison were examined for the presence of natural isolysins and heterolysins, in particular any heterolysins which might serve to distinguish cattle from bison. No antibodies of the latter specificity were observed.

All J-negative bison (table 3) possessed natural isolysins for J of bison and these also reacted with J of cattle. The activity of these natural J antibodies, as in J-negative cattle, varied from extremely weak to very strong from individual to individual.

In addition to the occurrence of natural anti-J, some of the bison (nos. 48, 59, and 57 in table 3) possessed natural lysins for cattle bloods of subtype U_1. Such antibodies are occasionally encountered in the normal serum of cattle (Stone and Miller, 1961).

The bison plasmas were also examined for the presence of heterolysins for the red cells of domestic sheep (*Ovis aries*) and for lysins which might distinguish group R from group O sheep. Ten of the J-negative bison and six of the J-positive bison possessed heterolysins for the red cells of all sheep. None of the bison possessed lysins specific for the red cells of group O sheep but all J-negative bison, as expected, possessed lysins specific for the red cells of group R sheep. (The R population of antibodies always includes anti-J, and both the J and R fractions are readily absorbed by sheep red cells of group R as well as by human red cells of groups A and AB.) The results were typical of those we have obtained in screening normal cattle serums or plasmas for anti-J, anti-R, anti-sheep O, and anti-human A, except in bison sera that we encountered no antibodies specific for the red cells of group O sheep. However, as pointed out by Sprague (1958b), natural antibodies specific for the red cells of group O sheep have so far been found only in one breed of cattle, the Hereford.

Some of the bison plasmas were examined not only for soluble J substance but also for the recently discovered soluble Oc substance (Sprague, 1958a). Three J-Oc phenotypes indistinguishable from three of the four types described by Sprague (1959a) in studies of cattle were encountered. These were the types J (soluble J but no Oc), JOc (soluble J and Oc), and Oc (soluble Oc but no J). None of the plasmas that were examined lacked both J and Oc activity. As in the case of JOc-positive and Oc-positive cattle, the red cells of JOc-positive and Oc-postive bison were not lysed by Oc antibody (specifically, natural bovine anti-sheep O).

The L System in Bison

The L system in domestic cattle is presently characterized by two phenogroups: L and its negative alternative "–", distinguished by tests with L reagent.

In the present and previous studies, all bison bloods were L-positive and the reactions were fully equivalent to those obtained in typing domestic cattle, thereby indicating, as shown in table 4, that the allele for L must be at or near fixation in the Oklahoma population of bison. However, as noted in the study of Owen et al., the red cells of one of 9 F_1 hybrids (cattalo) were L-negative thereby indicating

TABLE 4. *Phenogroups in the A, B, C, F–V, J, L, M, S, Z, and Z′ systems of bison blood groups and estimates of their gene frequencies in the Oklahoma population*

Genetic systems	Phenogroups	Gene frequencies	Known homologues in cattle	Known blood factors associated with each phenogroup
A	A_1D	0.22	Yes?[1]	A_1, A_2 and D[2]
	"–"	0.78	No	None
B	E_x'	0.25	No	P_x,[2] E_2'[2] and E_x'
	"–"	0.75	Yes	None
C	X_1	0.64	Yes	X_1, X_2 and NF12
	WX_1	0.22	Yes	W, X_1, X_2 and NF12
	C_2WX_1	0.14	No	C_2, W, X_1, X_2 and NF12
F–V	FV_b	1.00	No	F[2] and V[2]
J	J	0.55	Yes	J
	"–"	0.45	Yes	None
L	L	1.00	Yes	L
M	M_2	0.05	Yes	M_2
	"–"	0.95	Yes	None
S	S_b	0.21	No	S
	"–"	0.79	Yes	None
Z	Z	0.63	Yes	Z
	"–"	0.37	Yes	None
Z′	"–"	1.00	Yes	None (see Addendum)

[1] Homologues have not been compared in absorption tests.
[2] "Partial" cross reactions only with the respective cattle reagents.

that the allele for the negative alternative occurs in some bison.

The M System in Bison

As shown in table 3, five bison bloods cross reacted with antibodies in the M_2 reagent and the reactions were fully equivalent to those obtained in testing the bloods of domestic cattle of subtype M_2. As in cattle, the M_2 reactions of bison red cells (table 3) appeared to be independent of those obtained in the systems A, B, C, J, S, and Z.

It is concluded, therefore, that two alleles which control the phenogroups M_2 and the "–" alternative can account for the results obtained in typing these 56 bison with cattle M reagents. Estimates of the frequencies of the two alleles in the Oklahoma population are given in table 4.

The S System in Bison

The five reagents, S, U_1, U_2, U′, and H′ are presently used in typing the S system of domestic cattle and with these five reagents it has been possible to distinguish five phenogroups SH′, U_1H', U_2, H′ and a negative alternative designated "–". As may be noted by the symbolism used in designating these cattle phenogroups, the S-positive phenogroup (SH′) and the U_1-positive phenogroup (U_1H') are characterized in part by the fact that they both react with H′ antibodies. That is to say, we have yet to find in typing domestic cattle any S-positive or U_1-positive or SU_1-positive bloods which are negative with H′ antibodies.

In view of the association of blood factors **S** and $\mathbf{U_1}$ with blood factor **H′** in domestic cattle, we were particularly anxious to re-examine S-positive bison bloods with all available H′ reagents, since in the two previous studies there was no indication of an **H′** or **H′**-like blood factor in bison. For this reason, we used not only our pure H′ reagents but also incorporated

S + H′ and U_1 + H′ reagents (table 2) in the present study. The latter reagents can be converted to pure S and pure U_1 reagents by absorbing with cattle bloods of type H′ but cannot at present be converted to pure H′ reagents, by absorbing with cattle red cells, for reasons already indicated.

The results of the present study, as summarized in table 3, reaffirm the presence of an **S** blood factor in bison which seems to be fully equivalent to **S** of cattle. Nevertheless, there was no indication that bison red cells of any type will cross react with cattle H′ antibodies. The results are compatible with the interpretation that there are but two S phenogroups indicated in the Oklahoma population of bison: one designated S_b, which produces complete cross reactions with cattle S antibodies and also exhibits weak, partial cross reactions with one of our two U_1 + H′ reagents (see table 3), and the other designated "–", which like its homologue in cattle is non-reactive with any of the reagents presently used in typing the S system.

Estimates of the frequencies of the two alleles which control the S phenogroups of bison are given in table 4.

As a result of these studies, it is perhaps of some practical interest to point out that antisera or reagents which contain both S and H′ antibodies can be converted to H′ reagents by utilizing S-positive bison bloods in the absorptions.

The Z System in Bison

Two Z phenogroups, indistinguishable from their homologues in cattle, were previously recognized by Owen et al. (1958). The present data merely reaffirm those observations. The reactions obtained with Z reagents, including dosage reagents, are as summarized in table 3, and estimates of the frequencies of the two Z alleles in the Oklahoma population are given in table 4.

The Z′ System in Bison

The Z′ system in cattle is presently characterized by two phenogroups Z′ and its negative alternative "–". Phenogroup Z′ has proved to be either absent or rare in all breeds of *Bos taurus* so far studied.

As in the two previous studies, all bison of the present study were Z′-negative. Assuming that the Z′ locus is represented in bison, the only conclusion which can presently be drawn is that it is occupied solely by an allele whose product is non-reactive with Z′ antibodies. This allele has its homologue in cattle (see Addendum).

Discussion

In retrospect we have seen that some of the main points of difference in the blood-type comparisons of bison and cattle have either vanished or have considerably diminished. The first and perhaps foremost point, as set forth by Owen et al. (1958), was the virtually complete non-reactivity of bison bloods with 21 different reagents known to be reactive in the B system of cattle, an observation which indicated that the B locus is either absent in bison or is occupied solely by alleles whose products (phenogroups), like that of the allele designated *b* in cattle, were essentially non-reactive with the B reagents used in that study. We now know that neither of those indications was correct. The B locus is definitely represented in bison by at least two alleles, and the product of one of those alleles cross reacts not only with our facsimile E_3' reagents but also with two new B system reagents, namely, P_x and E_x'. Furthermore, one of those two alleles has what would presently appear to be its precise homologue in the allele *b* of cattle. The fact that we presently do not recognize a B phenogroup in cattle which is fully equivalent to E_x' of bison is not critical since the same situation occurs when breeds of cattle are compared. For example, phenogroup $BO_2Y_1A'E_3'G'$, whose controlling allele has a frequency of 0.42 in Milking Shorthorns and 0.03 in Holstein-Friesians, has so far not been encountered in our studies of Herefords (see Stormont, 1958).

The second point made by Owen et al. was the complete non-reactivity of bison bloods with H′ antibodies, in contrast to

the regular appearance of this blood factor with high frequency in equivalent samples of various breeds of cattle. Although this observation was reaffirmed by the data of Stone and Miller (1955) and by the present data, the significance of the point has been considerably reduced following recent observations made in this laboratory which have shown that **H′** is a member of the S system of cattle rather than a separate system by itself. It is certain that the S locus is represented in bison, in spite of the fact that there is no evidence in that species for blood factors related to $\mathbf{U}_1$, $\mathbf{U}_2$, **U′**, and **H′** of cattle. Even though no phenogroup equivalent to S_b of bison has as yet been detected in cattle, the fact that such a phenogroup occurs in bison makes us hopeful that its homologue may eventually be found in cattle.

The third point made by Owen et al. was the observation that all phenogroups in the C system of bison cross reacted strongly with reagents $\mathbf{X}_1$ and $\mathbf{X}_2$, while in cattle there are numerous C phenogroups which do not cross react with these reagents. The present data in no way detract from this salient feature; rather they add to it by the inclusion of still another reagent, namely, NF12, which cross reacted with all bison C phenogroups. However, in contrast with the two previous studies which reported positive reactions of all bison bloods with L′ reagent, none of six replicated L′ reagents of the present study produced reactions with bison red cells. The present results substantiate the opinion of Owen et al. that the previous L′ reactions were not of the nature of true cross reactions.

The fourth and final point made by Owen et al. was the indication of an allele F^{V_2}, controlling a phenogroup designated V_2, which would appear to be fixed in bison but has so far not been recognized in domestic cattle. We now know that the phenogroup produced by the bison F-V allele is partially cross reactive not only with V antibodies but also with F antibodies of various origin. Furthermore, we have been able to show that this particular bison phenogroup, designated FV_b in the present study, is capable of engendering antibodies which parallel the reactions of both F and V reagents in the cattle blood-typing tests. These differences between the F-V phenogroups in bison and cattle still stand as perhaps the most definitive differences between the two species. The question is, however, just how much longer will they stand? As already intimated elsewhere in this report, there is evidence for more than two alleles at the F-V locus of cattle and the product of one of the additional alleles might possibly turn out to be serologically indistinguishable from FV_b.

Actually, one of the most striking differences between the two species is probably not so much the allelic divergence at the various blood-group loci but rather the lack of heterogeneity in blood group alleles in bison, particularly at the B locus, as contrasted with the great heterogeneity in cattle. Several explanations could be advanced to account not only for the lack of diversity in bison but also to account for the extensive diversity in cattle but only one needs to be emphasized here, namely, the fact that our present populations of American buffalo have all been developed in modern times from foundation stocks of only a few animals (see discussion by Owen et al., 1958). We can never know precisely what the picture of bison blood groups was when literally millions of buffalo roamed the plains of North America, but better estimates can be made when we have more information not only on other herds of bison but also on blood-group heterogeneity in other wild species as contrasted with domestic forms that have arisen from them or have been closely associated with them in derivation. Hence, there is need to pursue much further the studies of blood groups in the subfamilies Bovinae, Caprinae, and Ovinae which are so richly represented by both wild and domestic species. Reed (1959) has expressed the opinion that comprehensive studies of blood groups in both wild and domestic

sheep would undoubtedly help clarify the taxonomic situation.

In their discussion of the reactions of bison red cells with cattle blood-typing antibodies, Owen et al. (1958) pointed out that there are no formal criteria whereby blood-typing antibodies developed for the purpose of differentiating individuals within one species, as cattle, may be used in differentiating species of the same or a different genus. We know, for example, that most of the bovine blood factors have significantly different frequencies in the various breeds of *Bos taurus*; and often of greater magnitude are the differences between breeds in the frequencies of alleles at each of the blood-group loci. This is particularly true for the complex B and C loci. We might expect, as mentioned by Owen et al., that there would be more extreme differences between species than between breeds or races within a species. We might also expect that some alleles, perhaps many or even all at any given locus, would have no precise homologues in related species of different genera but that their products would nevertheless exhibit some serological cross reactions. But, as the species under examination become even more distantly related it might be expected that the products of alleles at homologous loci would share fewer or no cross reactions. The key to certain of these events can be predicted merely by examining what has already been learned about allelic differentiation at such loci as B and C in cattle. Many pairs of contrasting B phenogroups or contrasting C phenogroups can be picked which do not share cross reactions with any of the presently known blood-typing reagents. If this can happen within a species, it must surely happen between species. The X-Z system of sheep (Rasmusen, 1958) is superficially homologous in many respects to the F-V system of cattle. Nevertheless, there is as yet no evidence for cross reactions between these two systems. This, however, does not exclude the possibility that homologous loci are involved.

The most desirable approach for comparing blood-group relationships of related species is that of using antibody-reagents which have been developed for the purpose of exploring individual differences in each of the species to be compared. In the bison-cattle comparisons we have not had available a battery of reagents developed specifically for bison even though a few of the reagents in the present study were derived from antisera directed against bison red cells. Therefore, one can only conjecture to what extent isoimmunization of bison would provide blood-typing antibodies which would distinguish further individual differences in bison and which might not cross react at all with cattle red cells. Some undoubtedly would cross react with cattle red cells and many of those that would can be predicted from what is already known about bison blood groups. On the other hand, if the present assay of blood groups in bison, as accomplished solely with cattle reagents, reflects most of the inherent blood-group variation in that species (and we are inclined to believe that it might), we could make some accurate predictions regarding the number of reagents that could be developed by the use of bison blood alone. We could also predict certain reagents now known to cross react with bison red cells which could not be developed, namely, X_1, X_2, NF12, F, L, and V simply for the reason that the genes for these blood factors seem to be fixed in bison. Bison isoimmune antiserum directed against such phenogroups as A_1D in the A system and E_x' in the B system would behave as monospecific reagents in the bison tests but should prove to be fractionable on absorption with appropriately selected cattle bloods. Indeed, if the present picture is a close approximation of the actual, the curious circumstance arises whereby reagents developed specifically for exploring blood groups in cattle would be just as useful as bison reagents in demonstrating individual differences in bison and at the same time would provide considerably more information on systems of bison blood groups as well as more information

on the serologic complexity of the phenogroups.

The question has arisen whether bison might possess one or more blood group systems not detectable by cattle reagents. All we can say at present is that there is little indication of such systems from the results of using cattle red cells in cross-absorbing both rabbit and cattle antisera against bison red cells. The antibodies in the rather low-titered "bison specific" reagents which have been developed from rabbit anti-bison serum can, for example, be attributed to phenogroup FV_b.

We believe that the answers to some of these questions will be forthcoming. In the meantime, it is established that at least 9 of the 10 blood group systems now recognized in domestic cattle are represented in bison. This observation, in conjunction with the observation that most of the blood-group alleles in bison (table 4) seem to have close homologues if not precise homologues in cattle, suggests that the two species are very closely related in evolutionary derivation, indeed, close enough in our opinion to justify their inclusion in the same genus.

In connection with another study (Stormont and Suzuki, 1958), we had the opportunity to test our cattle reagents with the red cells of one or more representatives of some other species (*Bubalus bubalis*, *Syncerus caffer*, *Anoa depressicornis*, *Boselaphus tragocamelis*, and *Taurotragus oryx*) in the subfamily Bovinae. However, in no instance was there any indication that any of these species stand near bison in the degree of blood-group relationship to cattle.

The only extensive comparisons of blood groups in cattle with those in a species other than bison, we believe, are the comparisons being made in this laboratory between the blood groups in domestic sheep (*Ovis aries*) and cattle. Serologic relationships have been established between the complex B and C systems of the two species and between the S system of cattle and the M system of sheep (Rasmusen et al., 1958; Rasmusen, 1960; Rasmusen et al., 1960; and certain unpublished data), and these relationships are in addition to the earlier known relationship between the J system of cattle and the R-O system of sheep (see Sorensen et al., 1954). But, in contrast with the bison-cattle comparisons, the sheep-cattle relationships extend only to the loci and not to the alleles. All the known phenogroups of the B, C, M, and R-O systems in sheep are clearly distinguishable from those of the equivalent systems in cattle. This also applies to the blood factors of the two species even though, for example, reagents which are identical in specificity in the cattle tests to such reagents as I, R, E_g', and NF6 (or B′) have been prepared from sheep isoimmune antisera. Another blood-type distinction, which has some taxonomic significance in artiodactyls (see Stormont and Suzuki, 1958), is that sheep are Forssman-positive whereas bison and cattle are Forssman-negative.

Summary

Data are presented on the results of blood typing 56 American buffalo (*Bison bison*) with 66 different cattle blood-typing reagents.

Evidence is presented which indicates that at least nine genetic systems of blood groups now recognized in cattle have their homologues in bison. These are the systems A, B, C, F-V, J, L, M, S, and Z. Comparisons are drawn between blood factors common to bison and cattle, and between the phenogroups in each of the systems of the two species. It is shown that most of the bison phenogroups and, by implication, their controlling alleles have closely similar or precise homologues in cattle. It is also shown that bison have soluble blood group substances serologically indistinguishable from the J and Oc properties of cattle. The same is true for the isohemolysins anti-J and anti-U_1 and certain heterohemolysins which occur naturally in the two species.

Certain differences between the two species, notably those encountered in typing the F-V and C systems, still persist and those differences are probably more ex-

treme than any so far encountered in comparing various breeds of domestic cattle. Furthermore, there is a marked reduction of heterogeneity in bison blood groups as contrasted with cattle. This is particularly true in the B system. Nevertheless, there are many striking resemblances that outweigh the few differences.

The over-all results indicate close taxonomic affinity of the two species. On the basis of the evidence on blood groups and on other observations mentioned in the introductory remarks of this report, further consideration should be given to the proposition that the American buffalo and domestic cattle deserve to be recognized as members of the same genus.

ADDENDUM

Since the manuscript was submitted, it has been observed in this laboratory that the rare bovine blood factor named Z′ actually belongs to the A system of bovine blood groups. This observation obviates the need to consider Z′ as representing a separate blood-group locus as was done in this paper. The blood-group loci which should be considered are A, B, C, F-V, J, L, M, S, and Z. On this basis it is clear that each of the 9 blood-group systems presently recognized in *Bos taurus* has a known homologue in American buffalo, thereby narrowing somewhat more the differences between the two species.

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THE EVOLUTION AND ECOLOGY OF CLOSELY RELATED SPECIES LIVING IN THE SAME AREA

JOHN L. HARPER[1]
WITH
J. N. CLATWORTHY[2], I. H. MCNAUGHTON[3], AND G. R. SAGAR[1]
Department of Agriculture, University of Oxford

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The diversity of both the flora and fauna of natural habitats has frequently been interpreted as the result of the intensity of a struggle for existence between nearly related forms. One of the most direct statements of this view was made by Darwin (1859) "... it is the most closely allied forms—varieties of the same species and species of the same and related genera—which, from having nearly the same structure, constitution and habits, generally come into the severest competition with each other; consequently each new variety or species, during the progress of its formation, will generally press hardest on its nearest kindred, and tend to exterminate them." This view (more recently expressed in various forms as "Gause's law" (Gause, 1934; Gilbert et al., 1952) has been challenged, for example, by Diver (1936) who stated "An examination of groups of closely related species shows ... that such species frequently live together in mixed colonies, i.e., in similar if not identical ecological conditions."

It might be expected that the question "Do closely related species live together?" could be answered by the direct examination of the floristics and faunistics of natural communities. However there there has been considerable controversy about the correct interpretation of field observations in this context.

Elton (1946) drew attention to the high frequency of genera represented by only one species in both animal and plant communities. An analysis of a number of plant communities showed that the average percentage of genera with only one species present was 84% and in a series of animal communities was 86%. Elton concluded that "this analysis confirms the general impression that some (although not necessarily all) genera of the same consumer level that are capable of living in a particular habitat at all, can co-exist permanently in an area; whereas it is unusual, in the communities analyzed, for species of the same genus to co-exist there."

Williams (1947) came to a quite different conclusion from a statistical analysis of the *same* community lists. Basing his argument on a statistical proposition about the relationship between categories and their members, called the logarithmic series and originally due to Fisher (Fisher et al., 1943), he concluded that "the diversification of species into genera is smaller in the small samples than in the larger. In other words, the smaller samples have fewer genera than would be expected in a random sample of the same number of species taken from the larger fauna and flora. This can only be interpreted as a natural selection—in the course of time—in favour of species in the same genus rather than against them."

The conflict of views found in the papers of Elton (1946) and Williams (1947) was examined by Bagenal (1951) who stressed the difficulties in interpreting "what is meant by the *same* habitat or the *same* community." He suggested that a confusion was based on differences in the meaning of the word "habitat" which in the sense used in mathematical models and derived experiments is a supposedly homogeneous system whereas in the communities chosen by Elton and Williams it has a much broader meaning. "From this standpoint Elton's conclu-

[1] Present address: Department of Agricultural Botany, University College of N. Wales, Bangor.
[2] Present address: Chiburo Agricultural College, Norton, S. Rhodesia.
[3] Present address: Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian.

sions appear to be correct, though based on unsuitable data, and Williams has provided the corollary that related species are more likely to be found in *similar*, though not identical, habitats than are unrelated ones" (Bagenal, 1951).

However, Williams showed that the relationship between numbers of monospecific genera, bispecific genera and so on in a community approaches very closely to, or is indistinguishable from, that obtained when the flora of a region which includes that community is sampled randomly. Furthermore the distribution of species per genus in a natural community obeys the same laws as the distribution of ticks per man's head or the numbers of scientific papers per scientist (Williams, 1944a, b, 1954). This conclusion is very disturbing if one believes that speciation and the separation of species into characteristic associations involves complex biologic processes. Of course the proof that the aggregation of species in a habitat is not a fortuitous assemblage of units, randomly chosen from the available range of species, is provided by the reality and reproducibility of natural succession. "Ecological succession is . . . important theoretically because it is one of the clearly established guarantees that communities have some orderly arrangement" (Elton and Miller, 1954). If we know from the evidence of succession that the selection of genera and species to fit a given habitat is not by random processes, the fact that the relationship between the occupants conforms with an expectation based on random sampling is surprising, but does not eliminate the problem of what laws govern the frequency of association of closely related species in nature. There would appear to be three prerequisites for the successful occurrence of closely related species together in the same area.

(1) The species must in the course of differentiation from each other have acquired differences in breeding behaviour—such that the interspecific differences remain distinct when the species are living together.

(2) The species must be able to tolerate the hazards which occur in the area in which they live together. In particular they must be tolerant of the regimes of frost and length of growing season, water supply (excess or deficit) and must tolerate any special chemical hazards such as toxic minerals or hydrogen ion concentration present in the area.

(3) The species must differ from each other in such a way that they do not enter into a struggle for existence in which one succeeds at the expense of the other.

It is necessary to establish at an early stage in this discussion what is meant by "living in the same area" and important to emphasize that there is a difference in the sense of "living in the same area" according to which of the above-mentioned three qualities of the species is being considered. If the investigator is concerned with the effectiveness of isolating mechanisms which prevent populations from interbreeding—then the distance between populations is obviously important. However pollen may be dispersed over greater distances than somatic material and there clearly exists in many cases the possibility of cross fertilization in nature between some species (particularly plants) which because of ecological separation, probably never engage in a direct struggle for existence. It has been found necessary therefore to define the areas occupied by two populations in a manner which distinguishes genetic from ecologic isolation. Cain (1953) argues "The important point is to decide whether the individuals (or their products) ever come into such proximity in their breeding seasons that all of the processes normally leading to fertilization could reasonably be considered possible. Let the word co-existence be used to denote such a state of affairs. Then sympatric species are those in which some individuals of each co-exist (in this restricted sense) without hybridizing,[2] allopatric species are those in which none co-exist."

Plant species may be isolated from each

[2] Cain later modified "without hybridization" to include cases in which hybrids are formed but do not contribute effectively to gene flow between the population (Cain, 1954).

other or may co-exist depending on the distance of effective pollen transport. Thus pollen of some *Pinus* spp. may be wind dispersed for many miles although Colwell (1951) has shown that most pollen falls close to the parent tree and Bateman (1947) emphasizes that the most likely cross pollinations are between close neighbours in both insect and wind pollinated species.

If an investigator is concerned with the ecological problems of closely related species living together the important point is to decide whether individuals (or their products) ever come into such proximity that a struggle for existence could reasonably be considered possible. Let the word "cohabit" be used to define such a state of affairs. This defines ecological association and so defines "the same area" for the ecologist just as "co-exist" is used to define an effective range of genetic interference for the systematist. The word "synecetic" is proposed for naming the relationship between cohabiting species, just as "sympatric" names the relationship between co-existing species.

The Co-existence of Closely Related Species

Species can only co-exist, *sensu* Cain (*loc. cit.*) if they do not merge by hybridization into one interbreeding population.

Systematically closely related species may arise in co-existence (sympatric speciation) or in geographic isolation (allopatric speciation). The presence of systematically closely related species in a state of co-existence may mean that they diverged in each other's presence, or that they migrated into each other's breeding range after diverging in isolation.

Geographic isolation is of overriding importance in the speciation of animals. "The view of Mayr (1947) is today generally accepted that apart from polyploidy, some type of geographical isolation is essential for the formation of distinct races" (Ford, 1954). The significance of this evolutionary theory to the ecologist is that closely related species of animal which co-exist are almost certain to have speciated in isolation.

Recently Thoday (1958) has demonstrated that divergence may occur within an interbreeding population if it is exposed to disruptive selection. He has shown, moreover, that the results of such selection may become more or less fixed. It is, however, not yet clear that this process would lead to speciation in nature as opposed to the stabilization of polymorphism.

In higher plants, however, speciation can undoubtedly occur within the effective breeding season and reproductive range of one population. Allopolyploidy (and perhaps autopolyploidy, obligate inbreeding and apomixis) offers a means by which individual plants may become reproductively isolated within an otherwise continuous or interbreeding population. This is only rarely possible in animals because the mechanisms of sex determination exclude the possibility of selfing or are disordered by polyploidy. Thus closely related species of plant which live in the same area may have arisen in each other's presence. Examples of such sympatric speciation in plants are widely known (see Stebbins, 1950; Darlington, 1956, 1958). A recent instance of sympatric speciation in the British flora by allopolyploidy involves *Senecio vulgaris* (2n=20), *Senecio squalidus* (2n=40), and *S. cambrensis* (2n=60). *S. squalidus* (2n=40) was introduced to botanic gardens in England, was recorded living on Oxford walls in 1794 and thence spread rapidly over the country (Druce, 1897). A sterile hybrid, between *S. squalidus* and *S. vulgaris* = *S. x baxteri* (2n=30) has been recorded quite often, and a fully fertile form, *S. cambrensis* with doubled chromosome number (2n=60) has been recorded from Flintshire, Wales. The parentage has been confirmed by deliberate hybridization and induced polyploidy (Rosser, 1955).

Because of the differences in the mechanisms of speciation available to animals and plants (and these comments relate specifically to higher animals and higher plants), we might expect to find closely related species of plants more commonly co-existing than closely related species of animals.

Moreover, the activities of man have made it far easier for plants to escape from geographic isolation than for higher animals, and so the chance of closely related and geographically isolated species being brought together is greater for plants than for animals. Seeds are small, often very small, dormancy may be long, and so accidental transport of seeds may occur readily in, for example, ship's ballast, in trouser turn-ups, in wool or in packing materials. Most plants are hermaphrodite or monoecious, and provided there is no perfect self-incompatibility mechanism, may set seed and reproduce their kind from one single colonizing individual. Most animals are dioecious and so two individuals, and on the average more than two will need to "escape" together in order to form a new colony (Baker, 1955). However, despite the special opportunities for speciation and migration in plants, there seems to be little difference in the distribution of number of species per genus in animal and plant communities.

The breakdown of geographic barriers between plant species, especially by the direct or indirect intervention of man, may bring pairs of previously isolated species into co-existence, and if it does so there is the opportunity for further speciation by hybridization and polyploidy. The ecological relationship which is established between such closely related species which migrate to become sympatric will be determined in part by the conditions of their origin. When such species meet they are likely to be differently adapted, because of differences in the forces of selection to which they were exposed during isolation. Such species are unlikely to make exactly the same demands on, or be equally tolerant of the hazards presented by, any habitat in which they meet. Lack (1940) points out that differences in the ecology of closely related species of birds living in the same area are likely to have originated as a result of the different conditions in the areas in which speciation occurred.

When a new introduction is made into the flora or fauna of a region, it need not necessarily, and will probably seldom, be into the habitat of its nearest relatives. Most aliens enter the flora of a region through gardens, ports and dockyards, or into agricultural land as contaminants of crop seeds (Brenan, 1947; Harper, 1957b). These "entry areas" are usually open plant communities where interference from other vegetation is relatively slight. In such sites the alien species is tested against the hazards of the new area and if successful in the new conditions (such as length of the growing season, perhaps a new photoperiodic condition, new pests or pathogens) it may produce seeds which are dispersed into other habitats. The habitats in which it is likely to be successful are more likely to be indicated by the conditions which the alien met during the period of its isolation and divergence, than by the habitat occupied by any already established related species. The success or failure of such an introduced species is determined by the extent to which it has already become preadapted to the conditions which it meets, by selection under the conditions which it had previously experienced. For example, *Senecio squalidus*, adapted to survive on the dry, well-drained soils of Sicily and Southern Italy, is preadapted to life on walls and railway banks in Britain. It is brought into an ecologic relationship with its congener *Senecio vulgaris* which inhabits disturbed ground, by the chance that stone walls commonly border on disturbed ground.

In contrast to the behaviour of species of allopatric origin, which have become secondarily sympatric, species of sympatric origin would most likely arise in an ecologic relationship with the parent species. The success or failure of the upstart species would then be determined from the beginning by its relationship to the parent populations. If the species were adaptively identical (probably only possible in theory), the upstart might remain in equilibrium with the parents, but if less well adapted to any of the sites occupied by the parents it would be unlikely to spread in them and might well be eliminated. It is presumably difficult for

an upstart plant species to escape from the habitats of the parents—an unsuccessful plant cannot run away from unfavourable conditions like an animal, and if it is less vigorous than its congeners in the habitat in which it arises it will be less able to reproduce and disperse itself into new habitats. (It is interesting to reflect that unfavourable conditions may stimulate animals to migrate, but will inhibit migration by a plant.) It can, therefore, be argued that sympatric speciation is likely to lead to the subdivision of the habitat occupied by parent species rather than to escape from their area.

If speciation occurs by allopolyploidy (polyploidy of a hybrid), then the new species may be better fitted to a habitat intermediate between those of the parent species than are either of the parent species themselves. In this case the new species could be said to be preadapted to, and would most likely arise near, such an intermediate habitat. Alternatively the new species may find itself in a part of the range of one of the parent species in which it is better adapted than the parent; this situation would permit the evolution of closely related species of plants occupying subtly different positions within a habitat. On the view that speciation following a sudden change in the breeding system is most likely to lead to extension of an existing range or the subdivision of a range previously occupied by one species, we would expect graded habitats to be most efficiently exploited by this process. It is, therefore, not surprising to find the members of polyploid series occupying stages in the zonation of plant communities. For example, "The British representatives of section *Glyceria* are paludal species; they occupy a wide range of habitats and are occasionally found growing together (Hubbard, 1942); nevertheless each shows slightly different ecological tolerances. *G. declinata* (2n=20) can grow in turf likely to be trampled and to dry out in summer. *G. fluitans* (2n=40) is ubiquitous, and like *G. declinata* is able to flourish in both acidic and base-rich soils, whereas *G. plicata* (2n=40) is intolerant of acid soils. Both *G. plicata* and *G. x pedicellata* (2n=40) are stoloniferous, forming large stands in open water. The hybrid flourishes in swift flowing water" (Borrill, 1958 a and b). The association of polyploidy with the zonation of species with altitude has also been pointed out (Clausen, Keck, and Hiesey, 1941).

It is also important in this respect that plant communities may be graded in time as well as in space; the natural succession of vegetation offers a strong selective force favouring those forms of a species and those species which can tolerate later stages in a succession. This would seem to offer an ideal opportunity for speciation and it is striking that closely related species are often represented in different stages of seres. Salisbury (1942) lists closely related species which illustrate this trend, and many of his examples form polyploid series.

If two well-differentiated populations, which have merited the rank of species by virtue of their morphological differences, meet and hybridize freely, the two populations may merge into one, and it would be reasonable to describe one or both of the species as being "hybridised out of existence." On genetic criteria for the validity of biological species, such species are not "good," even though where the populations are still isolated and there is no opportunity for hybridization they can be regarded as good morphospecies. It may sometimes be difficult to distinguish between a state in which both species merge and one in which a degree of introgression occurred during the process of extinction of one species, by the other (see discussion by Baker and Young in Lousley, 1955). An example of the disintegration of a difference between two species occurs where specimens of *Senecio cineraria* have escaped from cultivation and become sympatric with *Senecio jacobaea*. Colgan (1904) found two distinct types intermediate between the species, and Praeger (1951) that the range of intermediate forms had become enlarged.

The two oaks, *Quercus robur* and *Q. petraea* occupy fairly well-defined and dif-

ferent soil types in Britain. However, on some sandy soils, both species are able to establish in mixed stands, and fertile hybrids may be abundantly produced. But even when these two species are growing each on its characteristic soil type, considerable pollen interchange must occur. The failure of the species characteristics to be merged, as they are on the sands, must presumably be due to the adaptive inferiority of the hybrids in the parental habitats.

Baker (1948) has demonstrated that *Melandrium rubrum* and *M. album* exhibit both ecologic and genetic change consequent on hybridization when they meet. *M. album* is a weed of arable land which has spread from the Near East into areas occupied by *M. rubrum* where the woodland habitats of the latter species have been brought under agricultural management. When these two species meet, intermediate forms become established in ecologically intermediate areas and ultimately *M. rubrum* disappears leaving remnants of its genotype incorporated in *M. album*. This example illustrates vividly that the presence of closely related species living in the same area provides a problem which may only be solved in terms both of evolution and ecology

Three genetic situations may be roughly distinguished in the relationship between closely related species living in the same area:

(1) a breeding barrier prevents hybrids from being formed,
(2) a fertility barrier prevents hybrids from being fertile,
(3) fertile hybrids are formed. Questions of degree prevent these categories from being absolute.

If hybrids are formed but are sterile, they do not change directly the genetic character of the parent population, but they may well alter the ecological character of the parent population to an extent depending on (a) the frequency with which the hybrids are produced, (b) the habitat in which they develop, and (c) the extent to which they sterilize effective reproduction of the parent species. In some instances interspecific hybrids which are ± sterile are nevertheless more vigorous than the parents, e.g., hybrids between closely related species of *Populus* (Johnson, 1942), *Pinus* (Righter, 1946) and between *Papaver dubium* and *P. lecoqii* (McNaughton unpublished). When such sterile hybrids are capable of vegetative reproduction and are potentially long-lived they may spread and play the part of a distinct species ecologically, even though they may be inert genetically. The sterile hybrid of *Rumex obtusifolius* × *R. crispus* may colonize whole fields of grassland because it regenerates freely from root fragments. Love (1946) sowed mixed swards of three species of *Stipa* and found that interspecific hybrids were formed naturally and were more vigorous than the parents. The hybrid between *Circaea lutetiana* and *C. alpina* is quite sterile, but is successful and widespread because of its efficient vegetative reproduction (Gustafsson, 1946). The same is true of *Elymus condensatus* × *E. triticoides* (Stebbins, 1958). Timm and Clapham (1940) found that the sterile hybrid of *Juncus acutiflorus* (= *J. sylvaticus*) × *J. articulatus* was very widespread in Britain and that "under certain favorable conditions may be the most abundant jointed rush over wide areas."

Jones (1958) has found that a pentaploid race of *Holcus mollis*, presumed to have been derived from a hybrid *H. mollis* × *H. lanatus*, is the most common form in Britain. "The success of the pentaploid is attributed to its hybrid vigor in both growth and adaptability to soil moisture." Bradshaw (1958) found a similar situation on a smaller scale in which hybrids of *Agrostis tenuis* (2n = 28) and *A. stolonifera* (2n = 28) were better adapted than the parents to localized sites in grassland.

Hybrids which are ecologically intermediate between the parents may occupy zones which separate the parents in nature and provide a buffer preventing the mature parent species from coming into contact. In a habitat near Oxford the hybrid *Juncus acutiflorus* × *J. articulatus* "occupies a nar-

row zone between populations of *J. articulatus* on the river bank and of *J. acutiflorus* in a fairly dry meadow which is not heavily grazed" (Timm and Clapham, 1940).

In summary of this section it should be stressed that the frequency with which systematically closely related species are found co-existing is a function of (1) the mode of evolution (sympatric or allopatric), (2) the effectiveness of breeding barriers, (3) the chances of past migration, and (4) the heterogeneity of the habitats available.

The Ecologic Problems

The ecological problems which arise when closely related (or indeed unrelated) species are found together in the same area—under such circumstances that "all of the processes leading up to a struggle for existence could reasonably be considered possible," are expressed in a hypothesis of Gause (1934) "two similar species scarcely ever occupy similar niches." This statement has been variously reworded, e.g., "two species with similar ecology cannot live in the same regions" (Lack, 1947). (See also Anon., 1944; Allee et al., 1949; Williamson, 1949; Green, 1951; and Gilbert et al., 1952 for alternative statements of Gause's hypothesis.)[3]

Elton (1946) writes, "We simply do not understand exactly why populations of say a Pentatomid bug, a grasshopper, a moth caterpillar, a vole, a rabbit and an ungulate should be able to draw on the same common resource (grassland vegetation) and to remain in equilibrium at any rate sufficiently to form a stable animal community over long periods of years. . . . I think it has usually been assumed . . . that the equilibrium is made possible by some specialized division of labour, and that the animals do not come into direct competition at all; or else that the amount of resources is generally sufficient to provide for all the populations present because they are limited by factors other than food in the increase of their population." It is perhaps even more striking that we may find in a chalk grassland community sixty or more species of higher plants living together within an area of three or four square yards all requiring to share the light, water, nutrients and carbon dioxide in that area—yet forming a community of reasonable stability. In such an assemblage of species all have the power of exponential increase (whether by seed or vegetative spread), the species differ in biological characteristics but all demand the same qualitative constituents from the habitat, yet no one species is so superior to the others in exploiting the resources that it gains a monopoly of the habitat. Again Elton's comments on the structure of animal communities is relevant, "The ability of certain groups of species mostly separated by generic characters to exist in the same area while drawing upon a common pool of resources, is one of the central unsolved problems in animal community structure and population dynamics." The problem is more acute in plant populations because all the green plants in an area draw upon the same common pool of energy resources. The problem of two systematically closely related species existing together in the same area is only a special example of this general problem.

It may be taken as axiomatic that the influence of one species upon another in a struggle for existence increases as the density of the species increases. It is therefore extremely important to relate observations on a struggle for existence to the changing densities under which the struggle may occur. It is then possible to compare the reaction of species A and B to increasing density in pure cultures or stands with their reaction to each other in mixed populations.

Theories about the behaviour of ecologically closely related species living in the same area (synecetic species) derive mainly from studies of the dynamics of model populations—both mathematical models and simplified experimental systems (e.g., Gause, 1934; Park, 1955; Nicholson, 1954; Crombie, 1947).

[3] The literature on various zoological aspects of "Gause's hypothesis" is now so vast that it is possible only to select examples which illustrate the relevance of these studies to botanical investigation.

The Mechanisms of Population Control in Single Species Populations of Animals and Plants

The use of mathematical models has proved valuable in animal ecology for demonstrating the logical outcome of certain simple assumptions about the behaviour of populations. Such models have rarely been used in plant studies since Nägeli (1874), largely because the concept of a population of plants is much more nebulous than that of a population of animals. In communities of man and higher animals, the number of individuals gives a good indication both of the representation of a species in an area *and* of the potentiality for further increase *and* of the number of genetically independent units. The immense plasticity of individual plants and the powers of vegetative increase make it impossible for one parameter to do all of these things. A population of *Papaver rhoeas* may consist of individuals each bearing one flower and producing 4 seeds per capsule, or of plants each with about 400 flowers and 2,000 seeds per capsule. Clearly a count of the numbers of poppy plants gives little information about the population.

Moreover, numbers of individuals may give a wholly false picture of the success of a plant species. It is a very striking feature of the development of plant populations, that the highest density of individuals may be obtained under conditions poor for growth. This is shown in two examples from the work of Sukatschew (1928, quoted by Gause, 1934) who noted that the most fertile conditions supported the lowest numbers of fir trees per unit area. This superficially anomalous situation develops because on soil types supporting vigorous growth of fir, the more vigorous individuals quickly develop a canopy and the weaker members die—so that the stand is thinned to a relatively low density. This natural thinning is far less pronounced when individuals are stunted, e.g., by low nutrient status. In an experiment, Sukatschew demonstrated the same phenomenon with populations of *Matricaria inodora*. The proportion of the seedlings lost by natural thinning was highest in dense cultures, and the addition of fertilizers to the soil increased this mortality. A similar increase in percentage mortality with density is reported by Harper and Chancellor (1959) for *Rumex* spp. in pot experiments. These observations emphasize that the number of individuals does not give an efficient indication of the success of a plant species.

Of course even the counting of plants, though it may not need such elaborate techniques as counting animals, may be very difficult. The vegetative reproduction of plants provides the stumbling block in the way of counting individuals. Only by arbitrary means can it be decided when a grass tiller or an elm sucker has become an individual plant. No simple mathematical model can therefore be acceptable for theoretical studies of plant populations if by "population" is meant simply the numbers of individuals. However, it is an illusion to suppose that units must be counted before population dynamics can be studied.

The power of a population of animals to increase is usually a function of the number of individuals present—population models can therefore conveniently be set up with individuals as the units, and a rate of population growth which is potentially an exponential increase in the number of the units. In plants also the potential rate of increase is exponential—and in some exceedingly simple situations, such as the initial spread of an annual species in a habitat uniform in space and time it may be an exponential increase in numbers of individuals. In many perennial species there is superimposed upon the potential for increase of true individuals, the vegetative growth of clones which is itself potentially exponential.

Established populations may undergo profound changes, but these are usually insignificant in comparison with the potential rates of increase; "If an animal is introduced to a new and favourable area, it at first increases rapidly, but is soon checked, and thereafter its numbers, like those of other animals, fluctuate between limits that

are extremely restricted compared with what is theoretically possible. It follows that natural populations are in some way regulated, and that the controlling factors act more severely when numbers are high than when they are low" (Lack, 1954). The same reasoning holds for plant populations though not always in terms of numbers.[4] The ecologist is therefore led to look for factors which affect mortality or natality in attempting to explain the ways in which populations are controlled.

In plant populations there are two important phases at which there may be a reaction to increasing density (i) mortality affecting the number of survivors and (ii) plasticity affecting the size of and the number of seeds produced by survivors. In the case of annual species it is possible to consider the dynamics of populations in terms of seed numbers taking the view that the plant is simply the means by which one seed produces more seeds. Harper and Gajic (1961) have studied the dynamics of experimental populations of *Agrostemma githago* from this point of view and have attempted to show the ways in which mortality and plasticity of the plants act together to ensure (a) that seed output per unit area of pure stands is independent of seed input over a wide range of sowing densities and (b) that seed output per unit area from constant sowing density is strongly influenced by the presence of associated species. The population dynamics of *Agrostemma* and other annual species is also discussed by Harper (1960).

It is important to recognize that if the number of seeds which survive to produce mature plants is regulated—either because there is a limited number of safe sites for establishment, or because mortality increases with increasing density—no amount of excess seed production by the population will increase the number of plants present above a limiting value. The factors controlling population size will in this case be operating through mortality and may of course operate at different intensities in different years.

However, if the chance that a seed will produce a plant is unaffected by density, population control must occur through the plasticity of individuals. In vegetatively reproducing species, an initial population control affecting mortality of seeds and seedlings may become wholly hidden by later phases of vegetative multiplication. The vegetative multiplication of such species must be considered as an extended plasticity of individuals.

There are at least four important ways in which plant populations may be regulated—all of these have analogies in the regulation of animal populations.

(a) *Populations controlled by the number of "safe" sites available during recurrent catastrophes.* A population may be controlled because at some stage in its life cycle there is heavy mortality and only those individuals present in special sites may escape these hazards. Such populations might be said to be controlled by the number of such sites available.

Williamson (1958) has claimed that this mechanism may be responsible for the control of populations of woodland snails. During winter these are heavily predated particularly by squirrels. The number of snails surviving the winter is then a function of the number of sites (in this case hollows in the bases of trees) which are protected from predators during winter.

Plant populations can clearly be controlled in this same general manner. All seeds which land on the soil do not find conditions suitable for establishment. The size of a population of seedlings may then be a function of the number of seeds which land in sites suitable for seedling establishment. The suitability of a site for seedling establishment may depend on the extent to which seeds are hidden from predators or protected from pathogens, the extent to which

[4] We have deliberately avoided using any of the many available terms (e.g., density-dependent factors, negative density-dependent factors) for this phenomenon in view of the active controversy about their priority, logic and interpretation—see Solomon, 1958; Varley, 1957, 1958; Smith, 1935; Haldane, 1953.

the seeds are in a position to receive the necessary stimuli or sufficient water to germinate. Sagar (1960) has recently shown that subtle variations in the character of the soil surface may directly regulate the number of plants of *Plantago* spp. which develop from a constant density of seeds sown. The role of surface texture of the soil in determining plant numbers is also emphasized in studies of the regulation of populations of *Bromus* spp. (Harper, 1961).

It is characteristic of this mode of population control that the greater the seeding density the greater is the proportion of the population which fails to form established plants. The manner of control of population size may operate both by regulating the lower limits to which the populations are reduced as well as the upper limits which may be achieved. This is in contrast to the subsequently discussed control mechanisms which operate by regulating only the upper limits which a population may achieve.

(b) *Population controlled by a shortage of food* (a view supported by Lack, 1954, from observations on bird populations). It has frequently been demonstrated that the development of a plant population may be controlled by limiting supplies of light (e.g., Watson (1952) for field crops, Blackman and Rutter (1946) for *Endymion nonscriptus*, Hodgson and Blackman (1956, 1957) for *Vicia faba*, and Black (1958) for *Trifolium subterraneum*). Irrigation experiments have shown that water deficits may limit the growth of plant populations. Fertilizer experiments have clearly demonstrated the same type of effect and in particular the elegant experiments of Milton (1934 and 1940) show that the balance of species in upland *Molinia* or *Nardus* communities in Britain may be greatly changed and new species may enter if the levels of nitrogen, phosphorus, potassium or calcium in the soil are altered. In animal populations food shortage may increase mortality but in plants it most often operates through plasticity.

(c) *Populations controlled by interaction with predator or parasite populations* (a view supported by observations on insect populations, e.g., Varley (1947)). A classic demonstration that predators may control the balance of species in a plant community is found in the experiments of Jones (1933) who showed that various agriculturally desirable and undesirable combinations of species could be created from an initial mixed population by controlling the time and intensity of grazing by a predator (the sheep). Many comparable examples can be found in the agricultural literature, but studies on natural vegetation are extremely rare, badly documented, or derive from uncontrolled experiments. The role of fungi in the natural regulation of plant populations has been understressed—despite the fact that a great part of the seedling mortality of plants in the field may be caused directly or indirectly by plant pathogens such as *Pythium* spp., *Fusarium* spp., etc. (Harper et al., 1955; Harper 1955, 1956). Gibson (1956) showed that the percentage mortality of pine seedlings caused by damping off fungi increased with increasing density of sowing. The extent to which plant populations may be controlled by birds eating the seeds is virtually unknown, although some observations suggest that it may be profound (e.g., the main food supply of British wood pigeons in June is seed of *Ranunculus* spp., little else being eaten, while in February the leaves of *Ranunculus* form the bulk of the diet (Colquhoun, 1951)).

(d) *That population size is controlled by intrinsic species factors.* It is claimed that animals may react to increasing density by physiologic changes which affect reproduction. These changes may be independent of a shortage of food or the influences of predators and parasites (Chitty, 1957). The view has been developed fairly recently that the density of populations of plants may be controlled by inhibitory substances which are released from roots, shoots or seeds and which hinder or prevent the establishment of new individuals in the neighbourhood of an established plant. The evidence is at present fragmentary, and not

wholly substantiated, particularly for root excretions (review by Bonner, 1950), but strong evidence for population control by toxins washed out of leaves has been assembled for such species as *Hypericum perforatum* (T. O. Pritchard, unpublished), *Encelia farinosa* (Bonner, 1950) and for *Camelina sativa* which actively suppressed *Linum usitatissimum* (Grümmer, 1955). Grümmer gives a very full review of this type of plant interaction. Knapp (1954) has found interesting effects of high seed densities in depressing the germination of some species and this could presumably act as a mechanism of population control.

In contrast to these mechanisms of population control which involve a reaction to density it has been maintained that the control of populations is primarily by the direct action of environmental hazards to which the density of populations is irrelevant. This view is expressed among zoologists particularly in the works of Uvarov (1931), Andrewartha (1957), Andrewartha and Birch (1954), Birch (1957), and in the early writing of Bodenheimer (1938) who has more recently come to accept the generality of population control by reactions to density (Bodenheimer, 1958). It is difficult, however, to envisage that an upper limit to population size may be achieved in a manner independent of population density and theories involving recurrent catastrophes to account for variations in the upper limits of population size require that the size of a population always reflect the level to which it was reduced by the last catastrophe: this would not seem to account for the stability of natural communities, nor does it explain why extinction is not more common unless survival through the catastrophe depends on a limited number of safe sites as in (a) above. This problem is examined in detail for insect populations by Nicholson (1958).

The Regulation of Populations in Two Species Mixtures

If population control is by interference between individuals such that the population is recurrently thinned to a fairly constant low level as in (a) or because increasing density reduces the capacity for further increase (as in b, c, and d above) there is a real problem in the cohabitation of species.

The problem may be illustrated as follows: if into a stable community is released either of two populations of organisms, identical in all respects except that one population has been marked with a biologically neutral character enabling it to be distinguished (such as a harmless isotope of a normal body constituent), the two populations would be expected to show the same specific growth rate and to achieve the same maximum population size. If both populations were released together into the stable community, then the combined populations would be expected to show the same specific growth rate and maximum population size as either population alone. The two populations in this case are so similar (identical) that they behave in every respect as part of the same population. Any change in the relative proportions of the two populations must then be affected only by chance. In this idealized system, members of each population contribute equally to the density factors to which they respond equally; the individuals are wholly interchangeable and the effects of each individual and each population on the other are wholly reciprocal. It should be emphasized that the theoretical possibility of such balanced interference between species drawing on the same resources must always be allowed, e.g., Volterra (1926), Lotka (1925), Crombie (1947), and others.

From this idealized situation in which continued cohabitation of two populations is possible, influenced only by chance, there are two main types of departure caused by differences between the populations.

(a) *Two populations differing only in efficiency.* If two species occupy the same niche (position in the food chain) they may differ in the extent to which they are able to utilize this niche in population growth. A difference in the efficiency with which the species respond to a common controlling factor in the niche, e.g., the efficiency with

which food material is converted, predation is evaded, the proximity of neighbours is tolerated, or safe sites are occupied during catastrophes, would permit the more efficient population progressively to exploit the niche at the expense of the less able population. (Kostitzin (1939) gives an early and D'Ancona (1954) a recent review of models of such systems.)

The now classic experiments of Park (for a short account and bibliography see Park, 1955) on "competition" between the two closely related species of flour beetle *Tribolium confusum* and *T. castaneum* in culture showed that inevitably only one species survived from mixed cultures of both species thus confirming Gause's principle. More significant was the fact that under some combinations of temperature and moisture conditions the balance between the species was sufficiently delicate for probability to play a large part in determining the outcome and that the elimination of one of the species might take as long as 1,480 days (the shortest time in these experiments being 180 days).

Few experiments have been carried out along similar lines with plant populations although the experiments of Harlan and Martini (1938) on proportional changes in the populations of mixed barley varieties demonstrated the extent to which variations in reproductive capacity of varieties in different environments could lead to progressive changes in balance in the populations so that different varieties became predominant in different areas. A mixture of 11 different and common varieties of barley was sown in a range of localities. At the end of each year, seed was harvested and a random sample sown again in the following year. In nearly every site, the majority of the seeds at the end of the experiment consisted of one variety—which differed according to the locality. *The experiments lasted 4–12 years* and the outcome of this "struggle for existence" had not been wholly resolved at the end of any experiment. (Clements et al. (1929) report a series of somewhat comparable experiments mainly using transplants.)

In these terms it is important to stress not only that one of a pair of species will succeed in a uniform habitat at the expense of the other, but also that, if the species differ only slightly in their response to a common controlling factor, the struggle may take a long time to be resolved, and the outcome will become increasingly uncertain the more similar the species are in their response to the common controlling factor (see discussion of the development of mixed populations of *Lemna* spp. (Clatworthy and Harper, 1961)).

(b) *Two populations differing in the factors controlling their populations.* If two populations are controlled by different factors then it can be shown that continued cohabitation is possible. "For the steady state to exist, each species must possess some advantage over all other species with respect to some one, or group, of the control factors to which it is subject" (Nicholson, 1933). Thus, if the factor controlling population size (the controlling factor) were shortage of food material, two species could live together in equilibrium provided that they used different foods; if the factor controlling population size were predation, two species could live together if the predator differed; if the factor controlling population size were an intrinsic response to density, then two species could live together provided that they differed in intrinsic species response (always provided that the combined populations of the two species were not so great as to bring into action a different common controlling factor); if different types of site are required for successful persistence through recurrent catastrophes, then again continued cohabitation of the populations is possible. A statement of Haldane (1953) may be adapted to express this effect more clearly. "*The survival of the fittest will only cause the population of one (species) to increase at the expense of another if by fitness is meant the relative fitness of the (species) shown towards the same controlling factor.*"

The ability of two very similar non-interbreeding populations or species to live together in equilibrium is determined by the nature of their differences. If these are such that both species are still limited in population size as if they were part of one population (i.e., they have a controlling factor in common), then any difference in efficiency must inevitably lead to the elimination of one of the species or populations from the joint population. On the other hand, if species differ in such a way that their populations become independently controlled, continued cohabitation is possible, and the stable level of population of each species may not be altered if both are present together.

If it is clear from mathematical models that two non-interbreeding populations or species are unable to exist together in equilibrium if they hold a controlling factor in common (Williamson, 1957), the apparent cohabitation of two populations in nature presents a challenge. This may be met by showing (a) that apparent cohabitation is spurious because there is really a previously undetected heterogeneity in the habitat in space or time so that the populations do not really cohabit or (b) that the cohabitation is transient and does not represent a stable state, or (c) that the populations or species do not have controlling factors in common.

The conclusion that two or more species may persist together in equilibrium in a uniform environment only if they possess different controlling factors, focuses attention on the need to identify the nature of the factors controlling population size.

Spurious Cohabitation

Often the apparent cohabitation of closely related species is spurious, the habitat being in fact sufficiently non-uniform to permit partitioning amongst the species. For example, the three common species of *Ranunculus*, *R. bulbosus*, *R. acris*, and *R. repens* often appear to form an intimately mixed population of two or even three species in British grasslands. However, both experiments and field observations show that these species are in fact restricted to specialized microhabitats within the pastures (Harper and Sagar, 1953; Harper, 1957a, 1958). An essentially similar situation has been demonstrated for closely related species in the mixed *Sphagnum* communities of raised bog (Osvald, 1923, and Tansley, 1949). At any point in space on the raised bogs which were studied a continuous succession in time occurs from semi-aquatic *Sphagnum* species, such as *S. dusenii*, *S. cuspidatum*, and *S. cymbifolium*, in wet hollows through hummock formers such as *S. papillosum* and *S. tenellum* to species inhabiting better-drained positions as the hummock is raised such as *S. plumulosum* and *S. rubellum*. The changes in the Sphagnum species appear to control the associated vegetation until *Calluna vulgaris* assumes dominance and the *sphagna* at the top of the hummock die. In the meantime the regions between these hummocks have also undergone a similar succession: the lowest and dampest positions in the bog are therefore continually changing. The area occupied by each species of *Sphagnum is* continually migrating over the surface of the bog giving a superficial impression of over-all uniformity, but it is really a continually repeated pattern of succession at any one point in space. The whole area forms, therefore, a series of interspersed circles and cycles of regeneration and replacement of one closely related species by another. This example raises in extreme form the question of what is meant by two species living together in the same habitat. Within the center of each height zone in the hummocks only one species of *Sphagnum* is found and this zone may be in contact with other zones above and/or below which two species "come into such proximity in their growth and reproductive seasons that all of the processes normally leading up to a struggle for existence between them could reasonably be considered possible."

Obviously in such a raised bog community the fine gradations which exist from place to place offer just the conditions under

which a number of closely related species might be the most successful occupants; just because such species exhibit finely graded differences. Each lives in a different microhabitat within the community, kept within its narrow bounds either by precise adaptation to the conditions of the zone or by pressure from other species above and below or by a combination of both processes. We can define raised bog as the habitat and say that the many *Sphagnum* species cohabit within it; we can define height zones within the bog and say that each is a habitat in which closely related species cannot cohabit; we can define zones narrow enough to include not only those occupied by a single species but also the tension zones in which two species may be found. This type of problem arises in both plant and animal ecology and "the process of subdivision if carried to its logical conclusion brings us to a single species living in its chosen and (from the behaviour and life history point of view) unique habitat" (Elton and Miller, 1954). (See also discussion between Ross, 1957, 1958, and Savage, 1958, on the cohabitation of leaf hoppers.)

The Role of Differences in Nutrient Requirements in Permitting the Cohabitation of Species

It is seldom possible to demonstrate such a difference in the nutrient requirement of two plant species that their populations might be independently controlled, thus permitting the species to cohabit. However, one striking example of such a difference in nutrient requirement accounts largely for the ability of *Trifolium* spp. and grasses to form a more or less stable mixed community. On soils in which nitrogen is in short supply the growth of grasses may be controlled by this shortage. The clovers are independent of the external supplies of fixed nitrogen and their development is therefore independently controlled. However, if there is either application of nitrogen fertilizers releasing the grasses from their controlling factor, or cessation of grazing so that one controlling factor (e.g., shortage of light) operates on both grasses and clover, the grasses overwhelm the clovers in a struggle for existence.

A difference in the timing of nutrient requirements may also permit two species to cohabit. This is, however, most obvious where it is light rather than any specific chemical substance which is the controlling factor. The persistence of several species together is possible in a closed community if they make demands on light at different times so that their common controlling factor operates on the species independently. This behaviour is well illustrated in the division of growth periods in woodland communities into pre-vernal, vernal, and aestival phases—the various species making demands on the light supplies at different periods of the year.

While it is clear that differences in the nature or timing of nutrient or energy requirements may make two species sensitive to different controlling factors and permit two or more species to cohabit, the examples quoted are drawn from cohabiting species which are not taxonomically closely related. Indeed it seems most unlikely that the sorts of differences discussed above exist between closely related species. Nor is it at all likely that closely related species will differ in sensitivity to different toxins released by each other.

The Role of Parasites and Predators in Permitting the Cohabitation of Closely Related Species

The interspersion of two or more cohabiting species of plants may provide a degree of protection against the development of epidemics of disease or pests, if the species differ in the parasite or pest by which they are attacked. This protection develops because the spread of an infection or predator through a population is hindered by the interspersion of organisms resistant to this enemy. The following examples make this point clear.

(1) Where populations of wheat are badly damaged by physiologic races of black stem rust (*Puccinia graminis*) grain yields

can be raised by growing two different varieties of wheat interspersed, each of which is resistant to different strains of rust. Epidemics of rust races do not then develop rapidly in either wheat variety, because of the interspersion of potential host varieties with resistant varieties (Hanna, 1956).

The two closely related species of *Trifolium*, *T. repens* and *T. fragiferum*, are commonly found as wholly intermingled swards in lowland and riverside grasslands in Britain. The growth form and leaf shape of these species is very similar and the mixture of the two species offers a very striking example of cohabitation of closely related species. When plots of these species were grown as pure stands and as mixtures of the two species in experiments at Oxford, it was found that *Trifolium repens* was ignored by hares, which, however, completely defoliated the plots of *T. fragiferum*. In mixed stands *T. fragiferum* was ignored by the hares and the species persisted together (Clatworthy, unpublished).

It is perhaps suggestive that these differences between the response of species to controlling factors are found between closely related species. Clearly differences in palatability or resistance to fungal infection require less profound modifications in the biological equipment of species than would differences in nutrient demands or the timing of growth phases.

The Role of "Safe Sites" in Permitting the Cohabitation of Closely Related Species

In recent years much attention has been paid to the often subtle differences in the requirements of seeds of different species for germination. Differences in germination requirements are especially marked when comparisons are made of closely related species. *Ranunculus bulbosus*, *R. acris*, and *R. repens* germinate at different times in the year (Harper and Sagar, 1953). *Plantago major*, *P. media*, and *P. lanceolata* possess different requirements of light and temperature treatment before they will germinate (Sagar and Harper, 1960). *Avena fatua* and *A. ludoviciana* have different periodicities of germination (Thurston, 1956), *Papaver rhoeas*, *P. dubium*, *P. lecoqii*, *P. argemone*, and *P. hybridum*, which frequently cohabit in two, three or four species mixtures, all possess subtle differences in requirements for germination (McNaughton, 1960) which are not yet clearly understood.

The effect of different germination requirements among closely related species is that the species are unlikely to be able to germinate at the same time or under exactly the same conditions. Very small scale heterogeneity in the soil either in time or place may then permit two or more species to find "safe sites" at different times or places so that there is independent control of the number of plants of each species establishing in the area. Provided that each species sets sufficient seed to discover "safe sites" for the succeeding year, the interference between developing adult plants is largely irrelevant to the continued cohabitation of the species.

Harper and McNaughton (in preparation; brief account in Harper, 1961) have recently studied the mechanisms of population control in five species of the genus *Papaver* in pure stand and in all possible two-species combinations. In this genus each species responds to increasing density by increased percentage mortality. However, in 19 out of the 20 possible combinations of two species each species showed greater mortality in response to its own increasing density than it did to the increasing density of an associated species. The cause of mortality is not known in this case but it may be seedling blight which certainly occurred to some extent, or a difference in the precise site requirements for the germination of the seeds of different species in soil crevices. However, this example shows clearly that the populations of closely related species may be independently regulated.

Conclusions

It would seem that, provided it is accepted that populations of plant species are

regulated or controlled by a reaction to density, two or more species may only persist together in a habitat if their populations are subject to different controlling factors. The presence of two or more systematically closely allied species living in the same habitat must imply therefore that in the course of their evolutionary divergence these species have become subject to different controlling factors. Differences in site requirement for seedling establishment, differences in susceptibility to parasites or predators, differences in germination time or requirement for breaking dormancy seem to offer the kinds of differences between closely related species which might permit cohabitation and which might relatively easily be acquired during the course of species differentiation.

The frequency with which closely related species are found together in the same habitat is then a function of (a) the opportunities which have been available for the ancestral populations to become subdivided into independently evolving populations, (b) the opportunities for migration together after evolutionary divergence, (c) the chance that the meeting populations have acquired sufficient differences in reproductive behavior that they can co-exist without merging, (d) the likelihood that the populations after divergence are still tolerant of the major physical hazards of the area in which they meet, and (e) the chance that the differences which they have acquired during divergence are such that the species populations become independently controlled.

A cycle involving isolation, differentiation and divergence followed by migration together (and perhaps further sympatric speciation by hybridization and allopolyploidy) offers the opportunity for closely allied species to meet. The outcome of the meeting—either cohabitation of the species or a more or less rapid ousting of one by another—must then depend on the nature of the differences acquired during speciation. Those differences which result in the reproductive isolation of the species and those which place the species under different controlling factors may be both strengthened and modified after the meeting of the species.

Much of the interest which has been concentrated on the evolution of species has been concerned with the mechanisms by which reproductive isolation is achieved and fixed. This is, however, only a part of the process by which systematic diversity is developed. The continued persistence of the genetically isolated populations, except in geographical isolation from each other, must depend on a divergence in biological properties of the species such that they fill complementary and not mutually exclusive roles in vegetation. A major aim of this essay is to focus attention on the sorts of differences in the biology of higher plants which make them ecologically complementary.

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"OPERATIONAL NICHES" AND "COMMUNITY-INTERACTION VALUES" AS DETERMINED FROM *IN VITRO* STUDIES OF SOME SOIL ALGAE[1]

BRUCE C. PARKER AND B. L. TURNER

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Studies of the ecological niches of organisms are numerous, some of them classics in ecological literature, yet the differences in concept of the niche which pervade these studies are often as numerous as the studies themselves.

This investigation was undertaken originally with the intent of demonstrating, in part and insofar as possible, the approximate frequency and type of biotic coaction between algae and other microorganisms in a particular soil and for the purpose of elucidating some of the causal mechanisms involved.[2] It was not within the original scope of this investigation to apply the data to ecological niche relationships. However, during the analyses of the data, a simple calculation suggested itself, which seemed to have significance in demonstrating the approximate degree of similarity between the niches of two organisms. This calculation is described in this paper and is accompanied by a brief discussion of the ecological niche.

METHODS

Approximately 50 microorganisms, including algae, bacteria, actinomycetes, filamentous fungi, and protozoa, were isolated from a single sample of soil. The soil was collected aseptically in a steam-sterilized pint jar from a semicircular flower garden located in the traffic oval just west of the greenhouse of the Department of Botany, University of Texas. The ultimate origin of the soil, as far as has been determined, was the sandy alluvium from the bank of the Colorado River in the vicinity of Austin. Precautions were exercised throughout the isolation procedures to avoid contamination of the soil sample and cultures by foreign organisms.

Twenty-five of these original 50 isolates were selected for two-membered cultural studies. These are listed, as far as they have been determined, in table 1. In order to reduce experimental artificialities and to approach more closely the conditions of the natural environment of these organisms, soil from the original sample site was used exclusively as the basis of the culture medium, which was prepared as follows: 15-gram samples of the soil were placed in 125-ml Erlenmeyer flasks to each of which was added 60 ml of distilled water; the flasks were plugged with cotton and sterilized by autoclaving 15–20 minutes at 15 pounds per square inch. After the flasks had stood 24 hours or longer, studies of coaction were initiated. A typical experiment consisted of three such soil-water flasks inoculated with a pure culture of one organism; three more with a second organism; and still another set with both organisms, care being exercised throughout to deliver inocula of equivalent quantities. The flasks were stored in a culture chamber and exposed uniformly to about 300 ft-c intensity from cool, white, fluorescent, 40-watt lamps, on a 12-hour diurnal cycle of illumination, and at a constant temperature of 23° C. Two weeks after inoculation, cultures were removed, observed and tested for contaminating organisms. The organisms were observed macroscopically and microscopically for differences in amount of growth and in morphology, comparing the two-membered cultures with the

[1] The writers are indebted to Professor Harold C. Bold of the Department of Botany, University of Texas, for his many helpful suggestions leading to the completion of this investigation; also, to the National Science Foundation which made possible the support of this and other studies by the senior author under a grant to Professor Bold (NSF G-6373).

[2] The portion of the investigation dealing with the identification of the causal mechanisms is reported elsewhere.

TABLE 1. *Microorganisms studied in two-membered cultures*

Culture number	Major group	Identity
A-20	Chlorophyta	*Bracteacoccus* sp.
9	"	*Bracteacoccus* sp.
1b	"	*Bracteacoccus* sp.
10	"	*Chlamydomonas* sp.
2b	"	*Chlamydomonas* sp.
11	Chrysophyta	unidentified; resembles *Hantzschia* sp.
14	Cyanophyta	*Phormidium* sp.
B-1	Schizomycota	*Azotobacter chroococcum*
B-2a	"	*Nitrobacter* sp.
B-3a	"	*Nitrosomonas* sp.
B-6	"	unidentified; nitrate reducer
B-Az	"	unidentified
B-15	"	*Rhizobium leguminosarum* (from *Lupinus texensis* nodules)
Act-1	"	*Streptomyces* sp.
Act-2	"	*Streptomyces* sp.
Act-3	"	*Streptomyces* sp.
F-1	Deuteromycota[1]	*Alternaria tenuis*
F-2	"	unidentified; probably a basidiomycete
F-3	"	*Fusarium* sp.
F-4	"	*Penicillium frequentens*
F-5	"	*Cladorrhium foecundissimum*
F-6	"	*Pullularia pullulans*
F-7	"	*Cladosporium cladosporioides* and *C. avellaneum*; mixed culture
P-1	Protozoa	unidentified soil amoeba
P-2	"	*Colpoda* sp.

[1] The filamentous fungi were identified by Dr. C. W. Hasseltine.

pure-culture controls.[3] A reasonable number of these combinations were repeated (in triplicate) a second time without the occurrence of major differences from the previous results. A total of 143 two-membered combinations was examined, all involving at least one alga.

Several investigations of certain of these two-membered combinations were made to ascertain, if possible, to what degree the data might be regarded as "natural." These studies included: (1) the use of soil sterilized by ethylene oxide gas instead of by autoclaving; (2) the use of a special Pyrex U-tube apparatus which made possible the testing of two-membered combinations in association with the soluble, diffusible products of the entire soil community, etc.; and (3) studies of the effects of numerous climatic and edaphic variables, separately and in combinations, in two-membered cultures. Such studies supported the interpretation that many of the phenomena observed in vitro were probably to some degree of natural occurrence.

[3] The two protozoa were not examined in soil-water cultures, but rather on 1% soil-extract agar plates.

RESULTS

Space prevents the inclusion of numerous interesting morphological effects which resulted from the coaction of these organisms (Parker, 1960). Table 2 lists the qualitative growth effects for the 143 two-membered combinations examined in soil-water cultures. The scheme of Burkholder (1952) has been used to express the associative effects. They may be interpreted as follows: +, + = protocooperation (mutual benefit to both partners); +, − and −, + = parasitism (one partner stimulated in growth, but inhibits the other); +, 0 and 0, + = commensalism (one partner stimulated, the other unaffected); 0, 0 = neutralism (neither partner affects the other); 0, − and −, 0 = amensalism (one partner inhibited, the other unaffected);

TABLE 2. *Effects of isolates grown in 143 two-membered cultures in soil–water medium*[1]

	A-20	9	1b	2b	10	11	14
ALGAE:							
A-20	Bottom dweller; fair growth alone						
9	0, 0	Surface dweller; good growth alone					
1b	0, 0	0, 0	Surf–bottom dweller; good growth alone				
2b	−, −	−, −	0, 0	Diffuse; always motile			
10	0, 0	0, 0	0, 0	(+, 0)	Often at surface, and palmelloid		
11	0, 0	0, 0	0, 0	0, 0	+, −	Bottom dweller; near glass	
14	(0, +)	0, +	−, +	+, −	+, +	+, +	Trichome on bottom mostly
BACTERIA:							
B-1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
B-15	+, 0	+, 0	+, 0	(0, 0)	−, 0	+, 0	0, 0
B-3a	+, 0	+, 0	+, 0	−, −	+, +	+, +	+, +
B-2a	0, 0	0, +	+, +	−, −	−, 0	+, 0	0, 0
B-6	+, 0	+, 0	+, +	+, +	(−, 0)	0, 0	0, 0
B-Az	+, +	0, +	+, +	(0, +)	(−, 0)	+, 0	+, 0
ACTINOMYCETES:							
Act-1	+, +	+, +	+, +	−, +	+, +	+, +	+, +
Act-2	+, +	+, +	+, −	−, +	+, +	0, 0	+, 0
Act-3	+, −	+, +	+, −	−, −	0, 0	0, 0	0, 0
FUNGI:							
F-1	+, +	+, +	+, +	+, +	+, +	+, +	−, 0
F-2	+, +	+, +	+, +	+, +	+, +	0, 0	−, +
F-3	+, +	+, +	+, +	+, +	+, 0	0, +	−, 0
F-4	+, +	+, +	+, +	+, 0	+, +	+, 0	−, 0
F-5	+, +	+, +	0, 0	+, +	+, +	−, 0	−, 0
F-6	+, +	+, +	0, 0	+, +	+, +	−, 0	0, 0
F-7	+, +	+, +	0, 0	+, 0	+, 0	0, 0	0, 0
PROTOZOA:							
P-1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
P-2	0, 0					0, 0	0, 0

[1] Data represent estimates of relative growth from triplicate sets of flasks. For additional explanation see text.

and −, − = competition (mutual inhibition of growth). In table 2 the first term of a symbol (i.e., the term 0 in the case of 0, −) always indicates an alga; when two algae are involved, the first term of the symbol refers to the alga at the left in the vertical column and the second to the alga above on the horizontal axis. Symbols in parentheses indicate variability among the triplicate flasks and/or uncertainty of the relative numbers or quantities of one or both of the organisms involved; in these few cases a guess was made as to the nature of the coaction. Let us consider, for example, the symbol in the first column opposite B-3a; it is +, 0. This means that alga A-20 was stimulated in its growth (+) by bacterium B-3a, while the bacterium (B-3a) was unaffected (0); this symbolizes commensalism. It should be obvious that these results pertain only to specific experimental conditions and organisms and that they are qualitative.

Table 3 summarizes the data from table 2. It lists the incidence of protocooperation, commensalism, neutralism, amensalism, competition, and parasitism between algae and the different groups of microorganisms as well as for the entire group tested. A similar analysis is presented for stimulation, inhibition, and no effect, with respect to the algae only, as affected by their copartners. The following facts are demonstrated: (1) the incidence of algal stimulation and the incidence of protocooperation are greater than that of any other effect; (2) the incidence of inhibition of algae (amensalistic, parasitic, and competitive) is less than that of any other effect; and (3) cases of algal stimulation and/or pro-

TABLE 3. *Summary of analyses of data in table 2*

	Incidence	Incidence as %
Cases of Protocooperation (+, +)		
Algae associated with other algae	2/21	9.5
Algae associated with bacteria	8/42	19.0
Algae associated with actinomycetes	10/21	47.6
Algae associated with fungi	29/49	59.2
Algae associated with protozoa	0/10	0
Total associations	49/143	34.3
Cases of Commensalism (+, 0 or 0, +)		
Algae associated with other algae	3/21	14.3
Algae associated with bacteria	15/42	35.7
Algae associated with actinomycetes	1/21	4.8
Algae associated with fungi	6/49	12.2
Algae associated with protozoa	0/10	0
Total associations	25/143	17.5
Cases of Neutralism (0, 0)		
Algae associated with other algae	11/21	52.3
Algae associated with bacteria	13/42	31.0
Algae associated with actinomycetes	4/21	19.0
Algae associated with fungi	7/49	14.3
Algae associated with protozoa	10/10	100.0
Total associations	45/143	31.5
Cases of Amensalism (0, − or −, 0)		
Algae associated with other algae	0/21	0
Algae associated with bacteria	4/42	4.8
Algae associated with actinomycetes	0/21	0
Algae associated with fungi	6/49	12.2
Algae associated with protozoa	0/10	0
Total associations	10/143	7.0
Cases of Competition (−, −)		
Algae associated with other algae	2/21	9.5
Algae associated with bacteria	2/42	4.8
Algae associated with actinomycetes	1/21	4.8
Algae associated with fungi	0/49	0
Algae associated with protozoa	0/10	0
Total associations	5/143	3.5
Cases of Parasitism (+, − or −, +)		
Algae associated with other algae	3/21	14.3
Algae associated with bacteria	0/42	0
Algae associated with actinomycetes	5/21	23.8
Algae associated with fungi	1/49	2.0
Algae associated with protozoa	0/10	0
Total associations	9/143	6.3
Cases in which at least one alga was stimulated (+)		
Algae associated with other algae	8/21	38.1
Algae associated with bacteria	20/42	47.6
Algae associated with actinomycetes	14/21	66.7

TABLE 3 (Continued). *Summary of analyses of data in table 2*

	Incidence	Incidence as %
Algae associated with fungi	34/49	70.0
Algae associated with protozoa	0/10	0
Total associations	76/143	53.1
Cases in which at least one alga was inhibited (–)		
Algae associated with other algae	5/21	23.8
Algae associated with bacteria	6/42	14.3
Algae associated with actinomycetes	3/21	14.1
Algae associated with fungi	7/49	14.3
Algae associated with protozoa	0/10	0
Total associations	21/143	14.7
Cases in which at least one alga was apparently unaffected (0)		
Algae associated with other algae	8/21	38.1
Algae associated with bacteria	16/42	38.1
Algae associated with actinomycetes	4/21	19.0
Algae associated with fungi	8/49	16.3
Algae associated with protozoa	10/10	100.0
Total associations	46/143	32.2

tocooperation are most abundant in two-membered cultures which contain one alga (autotroph) and a heterotrophic microorganism (actinomycete, fungus, or certain of the bacteria). The last point seems to indicate a relationship with respect to the degree of similarity between the niches of the organisms; that is, under the experimental conditions, stimulation or protocooperation indicates the smallest degree of competitive inhibition[4] between the organisms involved. This observation led ultimately to the formulation of the "Community-Interaction Values" presented in table 5.

The "Community-Interaction Values" (C. I. Values) are derived from the data in table 2. The associative effects of two different algae were compared. A comparison of this sort is made in table 4 for two species of the genus *Bracteacoccus*, A-20 and lb. The number of instances in which the associative effects differed was noted; in the case of *Bracteacoccus* A-20 and *Bracteacoccus* lb, there were eight differences. This number was expressed as the numerator of a fraction in which the denominator represents the sum of the associations examined—i.e., both the similarities plus the dissimilarities. Thus, the comparison between A-20 and lb led to the fraction, 8/22, which is the "C. I. Value" for these two algae. Such values appear in table 5 for all the algae used in these studies.

DISCUSSION

If it is valid to assume that the smaller the number of dissimilarities (or the greater the number of similarities) between the biotic relationships of organisms, the more their niches have in common, then the "C. I. Values" ought to express the relative degree of similarity between the niches of any of the organisms. However, before discussing in detail the significance of these "C. I. Values" as here proposed, further clarification of the term "ecological niche," is essential.

Udvardy (1959) has sought to resolve the confusion between the terms, niche,

[4] "Competitive inhibition" should be distinguished from "toxic inhibition"; the former pertains to functional relationships and, hence, is tied in with the niches of organisms, while the latter is probably for the most part fortuitous.

TABLE 4. *Comparison of biotic coaction similarities and dissimilarities between* Bracteacoccus *A-20 and 1b*

	9	2b	10	11	14	B-1	B-15	B-3a	B-2a	B-6	B-Az
A-20:	0, 0	−, −	0, 0	0, 0	(0, +)	0, 0	+, 0	+, 0	0, 0	+, 0	+, +
1b:	0, 0	0, 0	0, 0	0, 0	−, +	0, 0	+, 0	+, 0	+, +	+, +	+, +
	Act-1	Act-2	Act-3	F-1	F-2	F-3	F-4	F-5	F-6	F-7	P-1[1]
A-20:	+, +	+, +	+, −	+, +	+, +	+, +	+, +	+, +	+, +	+, +	0, 0
1b:	+, +	+, −	+, −	+, +	+, +	+, +	+, +	0, 0	0, 0	0, 0	0, 0

[1] P-2 data were not included in the calculation of "C. I. Values" because of its incomplete nature.

biotope, and habitat. He has pointed out that the concept of niche, as originally proposed by Grinnell (1904) and later by Elton (1927), Gause (1934), and others is a "functional entity," not merely a place or space. Odum (1954) has described the ecological niche of an organism as the "profession" of that organism, in contrast to the habitat, which he analogizes with the organism's "residence." It is unfortunate that many previous discussions of the niche have been somewhat animal-centered and, therefore, have emphasized feeding habits (e.g., predator-prey relationships) and spatial relationships almost to the exclusion of other significant factors of the environment. Indeed, Udvardy (1959) has stated, ". . . since the definition of the niche (whether we take that of Grinnell, Elton, Gause, or anyone else) does not specify the number of essential factors or relations that compose the niche, there seldom will be an occasion to prove that two suspected species occupy exactly the same one." This should make clear the inadequacy of the many current conceptions of the ecological niche.

With these points in mind, and in an attempt to avoid further confusion in this paper, an "operational niche" is conceived as follows: *the "operational niche" of any organism represents the complement of the activities (active, reactive, and interactive) of that organism in a given habitat and biotope at a given time.*[5] The adjective "operational" is used in a sense similar to that of Mason and Langenheim (1957) for "operational environment." In other words, the "operational niche" is the instantaneous result of the effect of the operational environment upon the genetic potential of an organism. The terms habitat and bio-

[5] The "fundamental niche" and "realized niche," coined by Hutchinson in 1957, are seemingly identical to our "potential niche" and "operational niche"; however, we feel that our terms, which were borrowed in part from Mason and Langenheim (1957), are perhaps more useful, particularly as they are integrated with these authors' "operational environment" and "potential environment."

TABLE 5. *"Community Interaction Values"[1] for algal isolates studied*

	A-20	9	1b	2b	10	11	14	Total
A-20	—	3/22	8/22	13/22	11/22	15/22	18/22	68/154
9	—	—	10/22	12/22	11/22	15/22	19/22	70/154
1b	—	—	—	15/22	14/22	13/22	17/22	77/154
2b	—	—	—	—	15/22	17/22	19/22	91/154
10	—	—	—	—	—	12/22	17/22	80/154
11	—	—	—	—	—	—	13/22	89/154
14	—	—	—	—	—	—	—	103/154

[1] C. I. Value = $\frac{\text{Dissimilarities between association effects of one organism with those of another}}{\text{Total No. comparable examined associations}}$

Additional explanation in text.

tope have been included in this definition in order to emphasize their differences.

In these investigations, the "operational niche" of one organism includes the action and reaction of that organism in and to the microclimatic and edaphic (soil-water medium) environment operating in this admittedly artificial habitat (the flask) at any given time during the period of culture; it includes also the interaction (which in this case is coaction between it and its partner), for the actions and reactions of the partner may be equally important in modifying the "operational niche" of the first organism. Thus, the results of coaction which are obtained after two weeks of cultivation represent, for any organism, the summation of an infinite number of "operational niches."

The term "operational niche" may be more easily understandable when it is applied to the equation of competition presented by Gause (1934). The equation reads as follows: $\frac{dN_1}{dt} = b_1N_1\left[\frac{K_1 - (N_1 + aN_2)}{K_1}\right]$ where $\frac{dN_1}{dt}$, is the "rate of growth of the first species in a mixed population," b_1N_1 is the "potential increase of the population of the first species," and $\left[\frac{K_1 - (N_1 + aN_2)}{K_1}\right]$ (hereafter indicated by (x)) is the "degree of realization of the potential increase." It will be noted that b_1N_1 represents a *density-proportional* action, as defined by Odum (1954); thus, the organism would continue to multiply at rate b_1N_1 if there were no *density-dependent* factors or restrictions imposed upon it by the environment. In contrast, the term (x), is *density-dependent*, its value depending upon the number or biomass, or perhaps best of all the total activities, of the organisms involved; it is this term which can restrict the potential growth (b_1N_1). As Gause (1934) has used it, the value of (x) always lies between zero and unity, for only with such values can there be competition ("the struggle for existence"). If the potential growth (b_1N_1) is to be realized *at any one instant*, the value of (x) must equal unity. This would, of course, represent a condition of no apparent competition between the organisms involved, and the "operational niches" of the organisms (*at that instant*) ought to be entirely without interference. In order to obtain such a value of unity for (x), the term $-(N_1 + aN_2)$ must equal zero; this is possible only if a is negative to a degree sufficient to make $-(N_1 + aN_2)$ equal zero. If the value of a progresses beyond this negative value in a negative direction, then the value for $-(N_1 + aN_2)$ will become negative and will cause the value of (x) to exceed unity. This, in turn, will describe a stimulatory effect upon the species in question; that is, the potential growth rate (b_1N_1), which is based on the organism growing in pure culture, will be increased when joined with the particular copartner. Such a value, if existing only for a relatively few instants, may not be detectable.

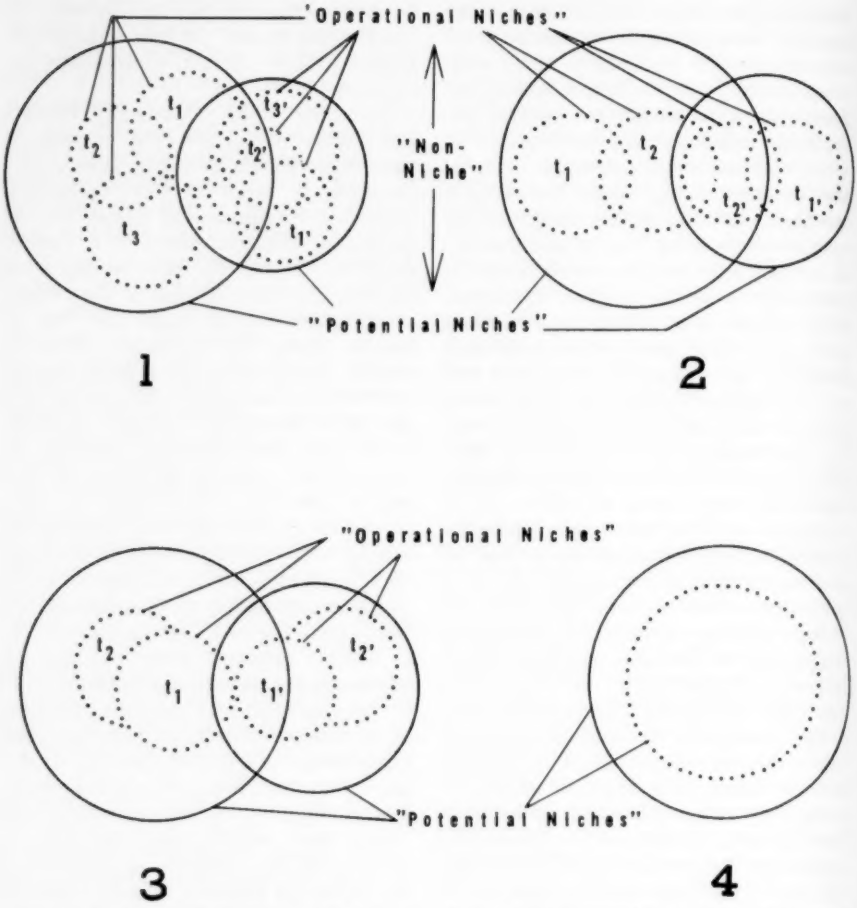
It should be evident, without further mathematical gymnastics, that the value for a may vary from one instant to the next. Indeed, Gause has stated: "The nature of the influence of one species on the growth of another does not remain invariable in the course of the entire first stage of competition." He showed that the more complex the environment, the more variable may be the value of a. Such variations in the values of a should be coincident with variations in the "operational niches" of the organisms concerned. In fact, the form of interaction with respect to each organism may change from stimulation to neutralism and from neutralism to competitive inhibition as a community develops. If a pertained strictly to habitat space, rather than to "operational niche," a negative value for it would be meaningless.

All this is indirect support of the view that the "operational niche" is a genuine quantitative entity which exists as a given time interval, in a given habitat or space, and includes the active, reactive, and interactive processes; the "operational niche"

describes the sum of the activities of an organism in a particular "operational environment." There should be also a "potential niche" which is determined by the genetic potential as affected by the "potential environment." But the "operational niche," while always within the limits of the "potential niche," rarely if ever operates in the latter to its full capacity in the natural environment. The "potential niche" is fixed for any organism and serves as the source of variability of the "operational niche" which is probably in a constant state of flux from one instant to the next. Following one step further in parallel with the reasoning of Mason and Langenheim (1957), there should be a third category, the "non-niche," which includes all functions or activities not contained within the "potential niche" of the organism.

Figures 1–4 illustrate in plane geometric fashion the hypothetical niches of two organisms in any common habitat. It is essential to realize that these figures *do not indicate space or habitat*, but rather the complement of biological activities as described in the definitions of "operational" and "potential niches"; these figures symbolize, in grossly over-simplified fashion, only a fragment of the infinite number of possible coactions which could occur hypothetically. A complete understanding of the figures, as interpreted by the writers, necessitates acceptance of the following: (1) an "operational niche" exists and is determined by the operational environment as the latter affects the individual organism; (2) the "operational environment" may change from one instant (t_1) to the next (t_2) and the "operational niche" changes concomitantly; (3) the boundary of an "operational niche" is limited by the boundary of the "potential niche"; (4) stimulatory effects (protocooperation, commensalism, parasitism) are explainable by a change in the operational environment which results in an increase in the capacity of the "operational niche"; and (5) inhibitory or competitive effects (parasitism, amensalism, competition) are explainable by interference (overlapping) between the "operational niches" of two separate organisms. More specific explanations accompany the figures.

Returning to the "Community Interaction Values" which are listed in table 5, their interpretation should be more understandable in terms of the "operational niches" of the algae and of figures 1–4. As previously mentioned, these "C. I. Values" ought to indicate the relative degree of similarity between the sum of the "operational niches" of one alga and that of another during the two-week cultivation period. For example, it was found that of 22 studies of coaction involving alga A-20 and alga 9 with other organisms from the community, three instances produced effects which differed for the two algae; this was indicated by a "C. I. Value" of 3/22. In the comparison between coactions involving alga A-20 and alga 14, however, the "C. I. Value" of 18/22 resulted, which indicates that 18 out of 22 associative effects were different. Therefore, as the "C. I. Values" approach unity (22/22), an increase in the number of dissimilarities in the associative effects of one alga with those of another is indicated, while as the values approach zero (0/22), the number of dissimilarities decreases. It seems probable that as these values approach unity, the "operational niches" (and probably to some extent the "potential niches") of the two organisms involved have less and less in common; also, as the values approach zero, the "operational niches" of the organisms involved have increasingly more in common; finally at zero (0/22), the "operational niches" ought theoretically to be identical, while at unity (22/22), they ought to be totally unrelated. In the latter case, the "operational niches" of one organism must lie entirely outside of the "operational niches" of the other *during the period under question*. It should be clear that in order to demonstrate incontrovertibly that the "operational niches" (or the "potential niche") of one organism are identical to those of another, an in-



Figs. 1–4. Hypothetical representation of the niches of two organisms during coaction. **1**, three operational niches for each organism, occurring at times t_1, t_2, and t_3. Since the "operational niche" of one organism does not overlap that of the other during any given time interval, the form of coaction is neutralism. **2**, two "operational niches" for each organism. Those occurring at t_2 overlap indicating a degree of competition between the coactants. At t_1 there is no competition. **3**, protocooperation. At t_1 the "operational niches" of the coactants do not overlap, but during this period each coactant produces products that stimulate the growth of the copartner during t_2. Thus the "operational niche" of each organism is enlarged through the activity of the copartner. **4**, two potential niches, one lying totally within the other. In this case complete competition is almost inevitable and the organism with the largest "potential niche" ought to win out permanently in the struggle for existence.

finite number of coactions may have to be examined under an infinite number of environmental variables.

The view that the relationships between any two series of "operational niches" (or perhaps between any two "potential niches") can be determined by studies of biotic coaction is not without supporting evidence from other studies. It has been borne out by studies of action and reaction

of the algae. Studies of (1) pH optima for growth, (2) preferential use of ammoniacal nitrogen, (3) ability to grow heterotrophically on glucose in darkness, (4) lack of demonstrable amylase and gelatinase activities, and numerous others demonstrate the similarities between the "operational niches" of the algae (Parker, 1960). The sum of these similarities and dissimilarities for each alga tends to coincide with the same for the results of coaction previously cited. Also, in general, the taxonomic similarities of the algae show relationships to the "C. I. Values." For example, the three species of the algal genus *Bracteacoccus* possessed values (3/22, 8/22, 10/22) most closely approaching zero, while values for the comparison between any of the green algae and the blue-green alga, *Phormidium* (14), tended to approach unity. There is, thus, corroboration in the taxonomic and morphological similarities between organisms; the more similar the algae (at least as determined by morphological criteria), the greater are the similarities between the "operational niches" and the closer to zero are the "C. I. Values."

The last column of table 5 contains the totals for the "C. I. Values" of the individual algae. The meaning of these total values is uncertain to the writers, but it would appear that the degree of similarity between the "operational niches" (or perhaps the "potential niche") of one alga with that of all the other algae taken as a hypothetical community is indicated. If these totals were representative of the entire natural soil community, they ought to have more significance.

Using the studies of action and reaction, it is possible to construct "Community Action Values" and "Community Reaction Values" which also indicate apparently the relationships between the "operational niches" of the organisms. However, "C. I. Values" may well be more reliable than those of action and reaction provided a suitable number of coaction studies are made. The reason for this is that the coaction between two organisms should represent a more balanced and complete summation of all aspects of the "operational niches," both quantitatively and qualitatively, because coaction is the result of all biologically significant active and reactive functions between two organisms combined.

There are, no doubt, numerous corrections and improvements which can be made regarding these ideas and methods of analysis. It is hoped that this brief discussion will, at least, serve as a stimulus to more experienced investigators to examine, to augment, and perhaps even to replace these thoughts and proposed calculations with others of superior quality.

Summary

Experimental studies of 143 two-membered cultures of soil algae with other microorganisms revealed numerous cases of interaction ranging from protocooperation to competition. Analyses of the data disclosed a simple numerical method which seems to express the relationship between the niche of one alga and that of another. This numerical expression is called the "Community-Interaction Value." Its application and potential use in ecological investigations are discussed in connection with the concept of the ecological niche, which is expanded to include an "operational," a "potential," and a "non-niche" for every organism.

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ISOALLELIC COMPETITION IN POPULATIONS OF *DROSOPHILA MELANOGASTER* CONTAINING A GENETICALLY HETEROGENEOUS BACKGROUND[1]

BENJAMIN HOCHMAN
Department of Genetics, University of Utah, Salt Lake City

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The *cubitus interruptus* (*ci*) locus in the small fourth chromosome of *Drosophila melanogaster* may be occupied by measurably distinct wild type alleles. Since these ci^+ genes in the homozygous state generally produce a normal phenotype of unbroken cubital wing veins, and therefore require special tests in order to be differentiated, they are termed wild type isoalleles (Stern and Schaeffer, 1943). By observing their effect in heterozygous combination with the mutant allele *ci*, these ci^+ isoalleles may be grouped broadly into two categories. At normal temperatures the members of one class of ci^+ isoalleles (class A) exhibit complete dominance over *ci* whereas alleles belonging to class B permit a number of ci^+/ci individuals to express the vein breaks which characterize *ci/ci* flies.

It has been reported previously (Hochman, 1958) that a chromosome which contains the class A isoallele ci^{+4} (or simply $+^4$) gradually replaces one carrying the class B isoallele $+^3$ in laboratory cage populations. In three separate population cages $+^4$ rose, within fifteen months, to approximately 90 per cent from initial frequencies of 30, 50, or 70 per cent. An increase from 20 per cent to 83.5 per cent was observed in a fourth population followed for ten months. Mathematical analysis demonstrated that $+^4/+^4$, of the three competing genotypes, had the highest adaptive value. Since the heterozygote $+^4/+^3$ did not display adaptive heterosis the eventual elimination of $+^3$ was predicted.

The genetic composition of the populations studied (one of limited genetic variability) may have been the decisive factor leading to the striking and unexpected difference in fitness between the two "normal" alleles $+^4$ and $+^3$ as well as the absence of euheterosis. In an attempt to confine genetic variation to the fourth chromosome all flies had been made isogenic for chromosomes I, II, and III derived from a highly inbred wild stock. The present investigation was undertaken to determine how two class B isoalleles might fare in individual competition with $+^4$ when the genetic milieu was more heterogeneous. In addition to $+^3$, a second class B isoallele, $+^5$, was tested against $+^4$, and in both series of experiments the genetic background contained variability in excess of that present in populations studied previously.

METHODS AND MATERIALS

Cages constructed of galvanized iron sheets (gauge 26) were used to house all of the experimental populations. A cage is illustrated in figure 1. Cage dimensions are length 15", width 11½", and height 6". Screening of brass strainer cloth (80-mesh) held in a frame forms the top cover of the cage. This frame is secured to the body of the cage by 20 short screws. The central portion of three sides of the cage (including the two long sides) is also screened. On the remaining short side there projects a funnel which can be plugged with cotton and cheesecloth and through which samples of living adults can be removed. Twelve circular holes (four rows of three holes each) are present at the bottom of the cage. Fitted tightly into these holes are rubber stoppers (size 12). These stoppers support stender dishes filled with approximately 30 cc of a cornmeal-sugar-agar-

[1] This investigation was aided by a grant from the University Research Fund, University of Utah.

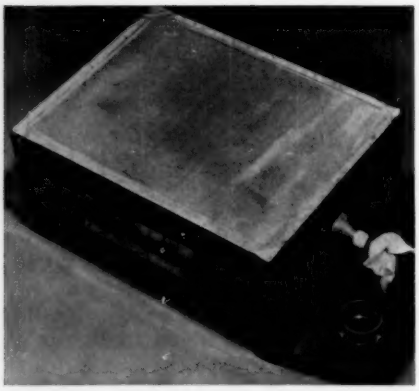

FIG. 1. The population cage used in the experiments. An empty food dish and rubber stopper are present to the right of the cage.

yeast-proprionic acid mixture. A thick suspension of living yeast cells is added to each food dish prior to its insertion into the cage. At 25° C a dish is kept in the cage for twenty days and when removed the dry medium contains little besides empty pupal cases. Census counts reveal that this population cage supports 3,000–5,000 living adults.

Eight experimental populations were studied. Four of these (cages 10, 11, 12, and 13) were maintained at the Research Division, City of Hope Medical Center, Duarte, California between February and July, 1957. Cages 14, 15, 16, and 17 were followed for a much longer period, April, 1958 to May, 1960 at Salt Lake City, Utah. With the exception of cage 17 which was kept at 20±1° C for the first twenty months, all populations were raised at 25±1° C. In December, 1959 cage 17 was placed in the 25° incubator and cage 16 was transferred to 20° C. Food dishes in any cage at the lower temperature remained for twenty-four days.

The three isoalleles studied, ci^{+3}, ci^{+4}, and ci^{+5} (hereafter referred to as $+^3$, $+^4$, and $+^5$, respectively) were found in the following phenotypically normal stocks. A laboratory strain of uncertain origin yielded $+^3$; $+^4$ was derived from an Iowa wild stock; and $+^5$ was discovered in a domesticated strain originated from flies trapped in Cockaponsett Forest, Connecticut. Dr. D. F. Poulson of Yale University kindly made the last named stock available.

Three strains, each homozygous for one of the isoalleles, have been maintained by the author for the past five years by the mass mating method commonly practiced in *Drosophila* stockkeeping. Judging from the history of these stocks there is little doubt that each is genetically heterogeneous in three of the four pairs of chromosomes. Theoretically the two fourth chromosomes of each strain are isogenic except for recent mutations. The F_1 individuals from a cross $+^4/+^4 \times +^3/+^3$ or $+^4/+^4 \times +^5/+^5$ would be expected to possess a large number of heterozygous loci.

The eight populations studied were each started with 100 F_1 females and 100 F_1 males from one of the two crosses just described. In cages 10, 11, 14, and 15 these 200 flies were all $+^4/+^5$. The original members of the populations raised in cages 12, 13, 16, and 17 were $+^4/+^3$. Frequencies of the competing isoalleles at the inception of an experiment were thus equal. Samples made at monthly or longer intervals provided an estimate of any changes in gene frequency that might have occurred in each population.

The sampling procedures have been described earlier (Hochman, 1958). In the work reported here appraisals of gene frequencies are based on 50 rather than 100 adult males removed from the population sampled. To compensate for possible sterility each sample contained 65 males which were individually mated to one or more $ci\ ey^R/ci\ ey^R$ virgin females. By observing the percentage of offspring (in each of 50 fertile vials) which display interrupted fourth veins, one can determine whether a tested male is homozygous for the class A isoallele, homozygous for the class B isoallele or heterozygous. The frequency of the $+^4$ gene in a sample is obtained by adding the number of heterozygotes to

TABLE 1. *Frequencies of $+^4$ observed in samples of four populations initially containing equal numbers of $+^4$ and $+^3$*

Day	Generation*	Population cage			
		12	13	16	17
0	0	.500	.500	.500	.500
30	2	—	.610	.550	.510
60	4	.610	.650	.700	.560 (3)
90	6	.620	.730	.750	.580
120	8	.680	.730	—	—
150	10	—	.700	—	—
180	12	—	—	.790	.600 (9)
210	14	—	—	.870	.730
240	16	—	—	.774	.600 (12)
270	18	—	—	.774	.616
300	20	—	—	.774	.663 (15)
330	22	—	—	.800	.648
390	26	—	—	.872	.752 (20)
540	36	—	—	.836	.762 (27)
570	38	—	—	.812	.762
600	40	—	—	.885	—
660	—	—	—	.894 (43)	.836 (34)
690	—	—	—	.918 (44)	.812 (36)
750	—	—	—	—	.872 (42)

* Generation time under cage conditions estimated at 15 days for cages 12, 13, and up to day 600 for cage 16. See text for an explanation of the numbers in parentheses.

twice the number of $+^4/+^4$ individuals and dividing the total by 100.

RESULTS

Experiments involving $+^4$ and $+^3$.—In each of the four populations studied it can be seen (table 1) that there is a marked increase of $+^4$ from an original value of 0.5. Populations 12 and 13 were of short duration but they reveal no essential difference in the pattern of change from that observed in cage 16. One detects in cage 16 an early rapid growth of $+^4$ to approximately 0.8 by day 180. There follows a period of slight or no change until the 600th day when the sample frequency registers 0.885. The final sample of cage 16 taken at day 690 places $+^4$ at 0.918.

A slower rate of increase is noted in cage 17. This population was kept for the first 600 days at a temperature of 20° C in contrast to 25° C for the other three cages. At the lower temperature the length of a generation is extended to about 20 days.

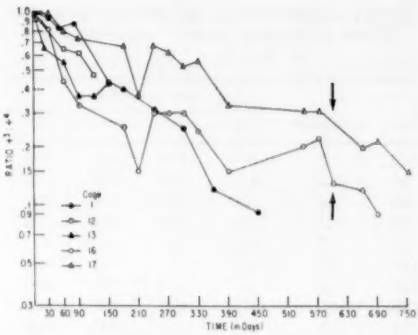

FIG. 2. Changes in the ratio $+^3 : +^4$ observed in samples of five populations. Ordinate is in logarithmic scale. The arrows indicate the time (day 600) at which cage 16 was transferred to 20° C and cage 17 was placed at 25° C.

The numbers in parentheses which follow certain of the cage 17 sample frequencies in table 1 indicate the probable generation tested provided that generation time was 20 days until day 600 and 15 days thereafter, when the cage was transferred to 25° C. Under this experimental regime there is a gradual change in the proportion of $+^4$-containing chromosomes from 0.5 to approximately 0.75 by the 390th day (generation 20). No further alteration in frequency is seen for 180 days. The next sample of the population was taken at about generation 34, four generations after the shift to the higher temperature. The $+^4$ isoallele at this time is gauged at 0.836 and the final sample (generation 42) measures it at 0.872.

As a second means of depicting the microevolutionary changes that occurred in the experimental populations the ratio of $+^3$ to $+^4$ in each sample was calculated. Initially this value was 1.0 in all cages. Semilogarithmic plots of the changes with time in this ratio for cages 12, 13, 16, and 17 are presented in figure 2. (This method of graphing was preferred because the curve in a semilogarithmic plot should approximate a straight line providing the adaptive values remain constant while a deviation from linearity suggests a change in fitnesses.) The curve of change obtained

TABLE 2. *Frequencies of $+^4$ observed in samples of four populations initially containing equal numbers of $+^4$ and $+^5$*

Day	Generation*	Population cage			
		10	11	14	15
0	0	.500	.500	.500	.500
30	2	.520	.570	.520	.550
60	4	.640	.480	.610	.590
90	6	.650	.720	.784	.640
120	8	.660	.680	—	—
180	12	—	—	.770	.660
210	14	—	—	.790	.760
240	16	—	—	.735	.748
270	18	—	—	.800	.800
300	20	—	—	.762	.693
330	22	—	—	.787	.748
390	26	—	—	.800	.775
540	36	—	—	.927	.872
570	38	—	—	.848	.905
600	40	—	—	.765	.885
660	44	—	—	.905	—
690	46	—	—	.916	.927
750	50	—	—	.825	.938

* Generation time under cage conditions estimated at 15 days.

in an earlier experiment (cage 1 in Hochman, 1958) is also graphed. It is evident from the figure that the rate of decrease of the ratio $+^3 : +^4$ is generally more rapid in cage 1 as compared to 16 and 17. The sample of cage 1 taken on day 450 (generation 30) appraises the ratio at 0.09. This low mark is not reached until the 44th generation in cage 16 and the lowest point for cage 17 is 0.15 for the 42nd and final generation. Moreover there is no period in the cage 1 curve similar to those times in cages 16 and 17 when the $+^3 : +^4$ ratio changed imperceptibly if at all.

Experiments involving $+^4$ and $+^5$.—An inspection of table 2 discloses that the $+^4$ isoallele increased in frequency at the expense of its competitor, $+^5$, in the four populations investigated. The findings in the short-term experiments (cages 10 and 11) agree basically with those encountered in the early periods of cages 14 and 15. In cage 14, $+^4$ climbed quickly from 0.5 to approximately 0.8 in six generations. It remained near this level for 300 days as the sample in generation 26 demonstrates. The population was not tested again until

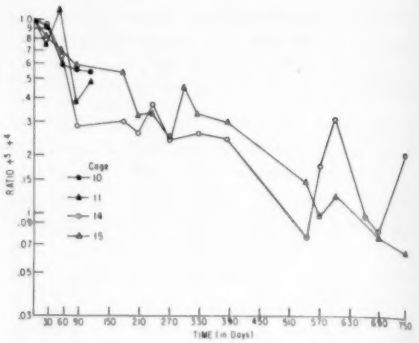

FIG. 3. Changes in the ratio $+^5 : +^4$ observed in samples of four populations. Ordinate is in logarithmic scale.

day 540 (generation 36) at which time $+^4$ registered at 0.927. In the five samples taken between the 38th and 50th generations the frequency of $+^4$ fluctuated considerably. At generation 40 the appraisal was 0.765; at generation 46 the estimate was 0.916. The final assay at the 50th generation measured 0.825. An average of the last six samples is 0.864.

The course followed by $+^4$ in cage 15 was not as erratic as that just outlined for the replicate experiment. There occurred in cage 15 a gradual growth in the number of $+^4$-carrying chromosomes from 0.5 to 0.76 by the 14th generation. This level was maintained up to generation 26. The next sampling of the population, made 10 generations later at the 540th day, placed $+^4$ at 0.872. Further increases in the frequency of the class A isoallele characterized the remaining four samples with the final appraisal at generation 50 reading 0.938.

A graphic presentation of modifications in the ratio $+^5$ to $+^4$ as calculated in each sample of the four populations is given in figure 3. The cage 14 curve shows an initial sharp decline in the ratio followed by a long stable period and culminated by marked variation about a level below that of the earlier stability. In cage 15 the decrease is more gradual, the period of no apparent change slightly shorter and the final half of the curve moves steadily downward.

Discussion

In separate competition with the class B isoalleles, $+^3$ and $+^5$, the class A isoallele $+^4$ behaved in a similar manner. Considering only those populations followed for two years one notes in all cases that $+^4$ became more numerous than its allele. These gains continued until the frequency of $+^4$ reached approximately the 80 per cent level. This point was achieved between the sixth and fourteenth generations at 25° C and sometime later at a colder temperature. There was subsequently an interval of from ten to twenty generations when no visible alteration of frequency occurred, a period of equilibrium. This state of balance was eventually disturbed and $+^4$ commenced a second term of increase. In three cages $+^4$ approached or passed 0.9 and was apparently continuing to advance when the experiments were terminated. This trend was not as obvious in the fourth cage although the final samples averaged 0.86 for $+^4$.

The pattern of increase, equilibrium and resumption of increase is distinctly unlike that encountered in earlier experiments. With the background isogenic $+^4$ grew at a uniform rate at the expense of $+^3$ and by the 30th generation was in the neighborhood of 90 per cent. Employing a method furnished by Dobzhansky and Levene (1951) it could be shown that $+^4/+^4$ was superior in fitness to the two other genotypes throughout the life of the population. A theoretical curve based on the adaptive values, W $(+^4/+^3) = 1.00$, W_1 $(+^3/+^3) = 0.95$ and W_2 $(+^4/+^4) = 1.10$ provided a close fit to the actual curve of decline in the ratio $+^3 : +^4$.

Attempts to find a single set of constant adaptive values to account for the occurrences in cages 14, 15, 16, and 17 have not been successful. In table 3 the observed changes in $+^3 : +^4$ or $+^5 : +^4$ are given and table 4 contains the theoretical ratio alterations assuming certain pairs of selection coefficients. (Due to the availability of a computing center at the University of Utah it was fairly simple to test many pairs of "W's." The six sets presented proved the most useful.) If $+^4/+^4$ possesses the highest fitness, as the first four columns assume, there will be no halting of the decrease in the ratio. On the other hand, the two columns on the right demonstrate that equilibrium is attained when the heterozygote is adaptively best.

Table 3. *Ratio of the class B isoallele ($+^5$ or $+^3$) to $+^4$ in samples*

Cage generation	Ratio	14 ($+^5 : +^4$)	15	16 ($+^3 : +^4$)	17
0		1.000	1.000	1.000	1.000
1		—	—	—	.960
2		.920	.820	.820	—
3		—	—	—	.785
4		.640	.696	.428	.723
6		.275	.562	.333	—
9		—	—	—	.667
10		—	—	—	.369
12		.298	.514	.266	.667
13		—	—	—	.612
14		.266	.316	.149	—
15		—	—	—	.515
16		.369	.333	.298	.539
18		.250	.250	.298	—
20		.316	.449	.298	.333
22		.266	.333	.250	—
26		.250	.298	.149	—
27		—	—	—	.314
28		—	—	—	.312
34		—	—	—	.196
36		.079	.146	.196	.231
38		.179	.104	.231	—
40		.307	.130	.130	—
42		—	—	—	.146
43		—	—	.118	—
44		.104	—	.089	—
46		.092	.079	—	—
50		.212	.066	—	—

A comparison of tables 3 and 4 discloses that an unchanged pair of adaptive values and the lack of euheterosis adequately explains the course followed in cage 17 only. Except for the tenth generation there are two combinations of W_1 and W_2, (.97, 1.05), or (.95, 1.03) which are satisfactory. It should be mentioned that the period of stability in the ratio of the competing isoalleles was in cage 17 the shortest of the four populations, lasting about nine generations.

There is little doubt that adaptive values were modified one or more times in the

TABLE 4. *Expected changes in the ratio* $+^3 : +^4$ *or* $+^5 : +^4$ *assuming certain pairs of constant adaptive values.* W_1 = *fitness of* $+^3/+^5$ *or* $+^5/+^5$; W_2 = *fitness of* $+^4/+^4$; *adaptive value of heterozygote* = *1*[1]

$W_1 =$	.970	.950	.950	.900	.600	.575
$W_2 =$	1.050	1.050	1.030	1.100	.900	.875
Generation						
0	1.000	1.000	1.000	1.000	1.000	1.000
1	.961	.951	.961	.905	.842	.840
2	.923	.905	.923	.819	.728	.726
3	.887	.861	.887	.741	.642	.643
4	.852	.819	.853	.671	.577	.580
6	.785	.741	.788	.550	.485	.494
9	.694	.638	.702	.410	.403	.418
10	.666	.607	.675	.372	.384	.401
12	.613	.550	.626	.306	.354	.374
13	.588	.523	.603	.277	.342	.364
14	.563	.498	.581	.251	.332	.355
15	.540	.474	.559	.228	.323	.348
16	.517	.451	.539	.207	.316	.341
18	.475	.408	.501	.171	.303	.331
20	.436	.370	.465	.141	.293	.323
22	.400	.335	.433	.116	.285	.317
26	.335	.275	.375	.079	.273	.308
27	.321	.262	.362	.072	.271	.307
28	.307	.249	.350	.065	.269	.305
34	.234	.185	.284	.037	.261	.300
36	.214	.168	.265	.030	.259	.299
38	.195	.152	.248	.025	.257	.298
40	.178	.138	.232	.021	.256	.297
42	.162	.125	.217	.017	.255	.296
43	.155	.119	.210	.016	.255	.296
44	.148	.114	.203	.014	.255	.296
46	.135	.103	.190	.012	.255	.296
50	.112	.085	.166	.008	.254	.295

[1] The author thanks Miss Dee Morgan of the Computer Center, University of Utah, for programming the equation used to obtain the values in this table.

other populations. The first six generations of cage 15 are best accounted for by .90 ($+^5/+^5$) and 1.10 ($+^4/+^4$). From generation 22 to 50 the pair, .95, 1.05 fits more closely. Between these periods there probably was a shifting of selection coefficients with possible heterozygotic superiority.

The results obtained in cages 14 and 16 are hardest to analyze. In both instances there were precipitous drops in the ratio and then an equilibrium period encompassing twenty or more generations ensued. A pair of W's, .575 ($+^5/+^5$) and .875 ($+^4/+^4$), lead to an equilibrium in the ratio $+^5 : +^4$ near the level of 0.29 observed in cage 14 from the 12th to 26th generations. However if these adaptive values had been the same from the inception of the population it would have taken nearly thirty generations to attain equilibrium. Next there is the problem of explaining generations 36 to 50. Assuming a new point of balance in the vicinity of 0.16 (the average of the six samples), W_1 and W_2 were again changed.

Concerning cage 16 and the stabilization of the ratio $+^3 : +^4$ at 0.26 between generations 12 and 22, a pair of adaptive values, .60 ($+^3/+^8$) and .90 ($+^4/+^4$) would have produced a balance near the level observed but not until more time had elapsed. The definite decline in the ratio which occurred in the final stages necessitates a change in the estimated fitnesses and probably indicates a loss of adaptive heterosis as well.

Does the microevolutionary pattern displayed by these populations deviate from that reported previously because of the relatively greater genetic heterogeneity prevalent in the more recently studied group? To conclude so would of course neglect or relegate to minor roles other factors of an external nature. Certainly variables such as time (over two years separated the experiments), climate (there are marked differences in humidity between the sites of the experiments), food (sugar replaced molasses) and even the utilization of metallic instead of wooden cages may have been partially responsible for the divergent results obtained. Unfortunately the data available do not permit an evaluation of the effects (if any) of these environmental agents.

One can however speculate about the importance of the amount of variability in the genetic background on the origin of a balanced polymorphic system involving the ci^+ isoalleles. The periods in the cage populations when the decline of the class B isoallele was arrested are believed to be due to the temporary establishment of a heterozygote selectively superior to either

homozygote. Why then should this have occurred in three of the populations described here but have failed to materialize in earlier experiments?

It is likely that $+^4/+^4$ usually has a higher adaptive value than either $+^3/+^3$ or $+^5/+^5$. The $+^4$ homozygote may even enjoy superiority over $+^4/+^5$ or $+^4/+^3$ under most competitive situations. Nevertheless, given a few generations and a rich supply of genic variability there may evolve an euheterotic heterozygote. This adaptive heterosis is probably not due entirely to heterozygosity at the *ci* locus but depends in part on interactions with other genes. Perhaps several combinations of modifying genes can serve as a milieu in which $+^4/+^5$ or $+^4/+^3$ is euheterotic. If this reasoning is accepted it follows that the higher the degree of heterozygosity present in the original members of a population the greater the chance that a balanced polymorphic system may emerge.

In addition to attempting to account for the establishment of equilibrium in cages 14, 15, and 16 there remains the problem of explaining the subsequent loss of said balance in two and possibly all three of the populations. Apparently $+^4/+^4$ regained or newly acquired a selective advantage over the competing heterozygote and the elimination of the class B isoallele was resumed. The loss of heterosis by a population as a sequel to its development is not unprecedented in *Drosophila* population genetics research.

Lewontin (1958) has experienced this phenomenon in cage experiments involving the Pike's Peak (PP) and Arrowhead (AR) gene arrangements in *Drosophila pseudoobscura*. In populations which had stabilized for fourteen generations at about 83 percent AR there took place a second rise in frequency of the predominant chromosomal type. At the conclusion of the experiments 22 generations later, AR registered near 93 per cent. Lewontin attributes this loss of heterosis to the constancy of external conditions which generally obtain in population cages. He reasons that an AR/AR combination possessing a higher fitness than the heterozygote may arise when environmental variations are of small degree. Lewontin explains both the origin and maintenance of balanced polymorphism in a population on the grounds that the heterozygous types have, on the average, greater homeostatic powers than do homozygotes. A homozygote may make the best adjustment in one or a few ecological niches but it is not generally as versatile as a heterozygote. However if a population resides in a relatively unvarying environment natural selection will favor the particular homozygote specialized for the prevailing conditions.

Whether or not Lewontin's explanation applies to the work reported here is a matter of conjecture. The fact that the experimental results are similar does not necessarily imply that the underlying mechanisms are the same. Yet the author has for some time noted certain resemblances between his experimental material and the intensively investigated gene arrangements in *D. pseudoobscura*. The *ci* locus is borne in a chromosome which undergoes little or no genetic exchange with its homologue. The fourth chromosome in *D. melanogaster* may be considered a block of genes as tightly linked as those included in the *pseudoobscura* inversions. One may distinguish different *melanogaster* fourth chromosomes by virtue of the ci^+ isoallele they carry just as the specific size and banding pattern in salivary chromosomes permits the discrimination of various *pseudoobscura* gene arrangements. For the investigator isoalleles or inversions act as good "markers." In either case however, any adaptive differences discerned in competition experiments probably depend not only on the particular marker present but are reflections of the total genetic material held together by the suppression of recombination.

Summary

Earlier work with laboratory cage populations of *Drosophila melanogaster* whose

genetic structure was relatively isogenic, demonstrated that the ci^+ isoallele $+^3$ declined at a uniform rate and tended to be eliminated in competition with $+^4$. Experiments reported here again involve $+^4$ versus $+^3$ as well as $+^4$ opposed by $+^5$ (phenotypically like $+^3$). In both experimental series the genetic milieu was more heterogeneous than had been the case previously.

Under the new set of conditions there was an initial increase of $+^4$ followed generally by a period of stability at a frequency near 0.8. The temporary existence of an adaptively superior heterozygote ($+^4/+^5$ or $+^4/+^3$) is believed responsible for the equilibrium. Mathematical analysis indicates that selective coefficients changed one or more times in most of the populations in contrast to the constant adaptive values which characterized the isogenic groups.

The postulated balanced polymorphism was subsequently disturbed and $+^4$ began to climb again at a rate which could have led to the loss of its allelic competitor. It is suggested that the degree of heterozygosity in the genetic background may be an important factor in the origin of a balanced polymorphic system. The eventual breakdown of equilibrium parallels the experiences of Lewontin with two different gene arrangements in *D. pseudoobscura*. His explanation that heterosis may be lost under constant environmental conditions may also apply to these investigations.

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MORPHOLOGICAL AND CYTOLOGICAL VARIATION IN *HELIANTHUS PETIOLARIS* WITH NOTES ON RELATED SPECIES[1]

CHARLES B. HEISER, JR.
Department of Botany, Indiana University, Bloomington

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Helianthus petiolaris Nutt. is an annual sunflower of wide distribution in sandy areas of the central and western United States. Most taxonomists have recognized two taxa within the species, *H. petiolaris* proper, and *H. petiolaris* var. *canescens* A. Gray, although some have considered the latter a distinct species, *H. canus* (Britton) Wooton and Standley, and it is so treated here. The present author (Heiser, 1958) recognized two subspecies, *H. petiolaris* ssp. *petiolaris* and *H. petiolaris* ssp. *fallax*, in what previously had been treated as the typical element. In the present paper, the morphological variation and geographical distribution of *H. petiolaris*, the cytogenetic analyses of hybrids within the species, and the results of hybridization with other annual species are considered.

Geographical distribution. — The map (fig. 1) has been compiled from the herbarium specimens from Gray Herbarium, Indiana University, Inter-Mountain Herbarium, Missouri Botanical Garden, United States National Herbarium, and the University of Wisconsin. It can be seen that *H. petiolaris* ssp. *petiolaris* has its greatest concentration in the southern Great Plains,[2] which probably represents its pre-human range. It is now also fairly well established in the Central Plains. In Indiana, where excellent records are available (Deam, 1940), its spread since 1900 has been observed. In the East it occurs as an adventive weed and it is now well established in some areas in the Southeast. There is a discontinuity in the western part of the range, and it is again found in three areas in California.

Helianthus petiolaris ssp. *fallax* is limited to more arid regions and is found only in western Colorado, eastern and southern Utah, New Mexico, and Arizona. The two subspecies intergrade to some extent on the Colorado–New Mexico and New Mexico–Texas borders, and it is not always possible to assign subspecific names to herbarium specimens from these areas.

Helianthus canus ranges from West Texas to California (area shown in outline on map). The distribution of the recently described *H. neglectus* Heiser, a close relative of *H. petiolaris*, limited to a small area of West Texas, is indicated on the map. Hybrids between *H. annuus* L. and *H. petiolaris* for which there are herbarium vouchers are also plotted. *Helianthus annuus* has an even wider distribution than *H. petiolaris*, and although the two species have distinct soil preferences they are frequently found growing adjacent to each other.

Morphological Variation.—Some idea of the regional variation can be gained from figure 2, in which seven characters have been used to construct polygons for each of the populations grown in the field at Bloomington in 1957 or 1958. The mean values for number of phyllaries, "phyllary index," "leaf index," number of rays, and the percentage of plants showing the absence of leafy bracts, presence of purple anthers, and absence of long trichomes on the peduncles are represented. The number of plants grown in each population and

[1] This study was carried out with the aid of grants from the National Science Foundation. I should also like to express my thanks to Dr. R. E. Cleland and Dr. G. L. Stebbins, Jr. for reading portions of the manuscript, to the numerous individuals (see table 1) who supplied seeds, and to the students who helped with various phases of the investigation. Grateful acknowledgment is also made to the curators of the herbaria who have made material available for study.

[2] It is also recorded from Oregon and Idaho but exact localities could not be ascertained.

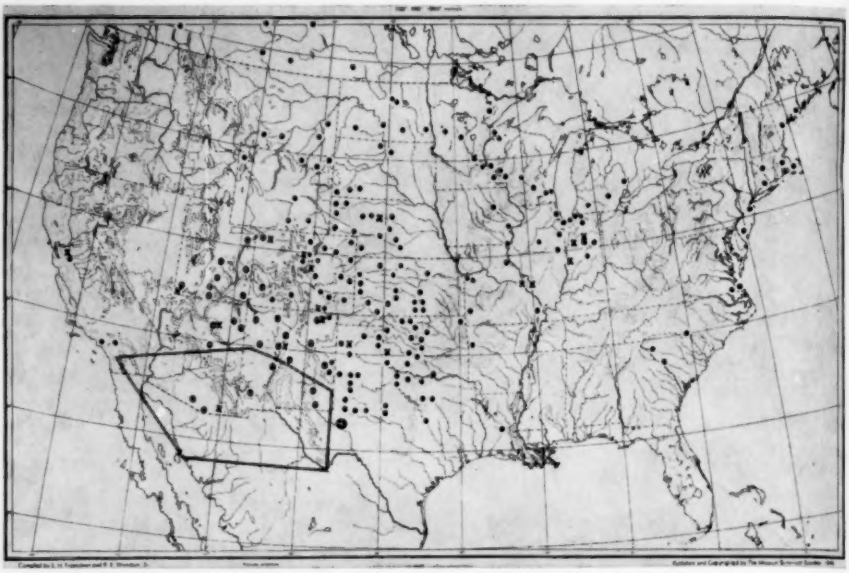


FIG. 1. Distribution of *H. petiolaris* ssp. *petiolaris* (solid dots), *H. petiolaris* ssp. *fallax* (open dots), hybrids of *H. annuus* and *H. petiolaris* (x), *H. neglectus* (triangle). The generalized area of the distribution of *H. canus* is shown by the hatched lines. (Base map used through the courtesy of Dr. R. E. Woodson, Jr. and the Missouri Botanical Garden.)

their sources are given in table 1. A total of 11 characters was measured or scored and means and standard deviations were computed. However, the graphic method of portraying the results of the study of seven characters is considered more satisfactory than a table giving all the measurements. Although it may not be possible to read the exact value for the characters from the polygons, it is possible to make general comparisons quite readily. The populations grown from Wyoming and Canada are not included since they were considered quite atypical from the plants in nature. Many of the plants from these areas died before flowering, and those which survived usually produced only a single small head at a height of 15–25 mm and came into bloom two weeks to a month earlier than plants from other populations. Similar results had been secured previously with *H. annuus* derived from high altitudes and northern latitudes (Heiser, 1954).

Analysis of the morphological features reveals the following:

1. The mean *number of phyllaries* ranged from a low of 16 in *New Mexico 31* to a high of 41 in *Oklahoma 23*. The populations of ssp. *fallax* generally have fewer phyllaries than do plants of ssp. *petiolaris*. The higher numbers in general come from Texas and Oklahoma.

2. A low "*phyllary index*" is also evident in the populations from New Mexico, Arizona, and Utah. The index values ranged from a low of .14 in *Arizona 37* to a high of .44 in *Colorado 13*. The index was computed by dividing the width of the phyllary by its length. Plants of ssp. *fallax* generally have phyllaries 3 mm or less wide, whereas plants from other areas frequently have phyllaries over 3 mm in width. The length of phyllaries of the southwestern plants is quite variable, but frequently is 15 mm or more, whereas the average length for plants from other areas is 11 mm.

TABLE 1. *Source of Material of* Helianthus petiolaris

Locality		Collector	n*
1. South Carolina	Edgefield Co.	D. M. Smith 72	7
2. Minnesota	Dakota Co.	Heiser 3255	10
3. Indiana	Lake Co.	C. Dale	17
4. Indiana	Vigo Co.	Heiser s.n.	19
5. Illinois	St. Clair Co.	Heiser 5526	22
6. Missouri	St. Louis Co.	Heiser 5404	25
7. Kansas	Pawnee Co.	P. Weatherwax	22
8. Kansas	Wallace Co.	Heiser 5407	
9. Kansas	Kingman Co.	Heiser 4176	10
10. Kansas	Ford Co.	Heiser 4175a	
11. Colorado	Douglas Co.	B. & D. Norby	
12. Colorado	Lincoln Co.	Heiser 4549	6
13. Colorado	Grand Co.	Heiser 4544	12
14. Colorado	Bent Co.	Heiser 4175	11
15. Colorado	Bent Co.	Heiser 5649	18
16. Colorado	"eastern Colorado"	M. Bell	24
17. California	Los Angeles Co.	Heiser 773	
18. California	San Bernardino Co.	J. & L. Roos 5199	9
19. Oklahoma	Marshall Co.	F. Carter	
20. Oklahoma	Beckham Co.	W. P. Stoutamire	14
21. Oklahoma	Beckham Co.	Heiser 4503	18
22. Oklahoma	Oklahoma Co.	Heiser 4109	18
23. Oklahoma	Oklahoma Co.	Heiser 4501	16
24. Texas	Wheeler Co.	Heiser 4111	21
25. Texas	Wheeler Co.	Heiser 4504	20
26. Texas	Hale Co.	Heiser 4113	14
27. Texas	Lynn Co.	Heiser 4117	19
28. Texas	Dawson Co.	Heiser 4120	20
29. New Mexico	San Juan Co.	Heiser 4159	3
30. New Mexico	Sante Fe Co.	Heiser 5658	10
31. New Mexico	Colfax Co.	Heiser 4171	5
32. New Mexico	Quay Co.	Heiser 4508	19
33. New Mexico	Guadalupe Co.	Heiser 4509	16
34. New Mexico	Valencia Co.	P. Weatherwax	
35. New Mexico	Valencia Co.	P. Weatherwax	
36. Arizona	Santa Cruz Co.	Leslie Gooding	21
37. Arizona	Cochise Co.	F. N. Young	5–21
38. Arizona	Apache Co.	Heiser 4148	
39. Arizona	Apache Co.	Heiser 4149	
40. Arizona	Coconino Co.	Heiser 4151	
41. Arizona	Navajo Co.	Heiser 4162	10
42. Arizona	Navajo Co.	Heiser 4165	5–11
43. Arizona	Coconino Co.	P. Weatherwax	
44. Utah	Utah Co.	D. D. Ruesch	
45. Utah	San Juan Co.	Heiser 4155	
46. Utah	Kane Co.	Heiser 4513	7
47. Wyoming	Platte Co.	W. P. Stoutamire 2641	
48. Canada	Saskatchewan, Swift Current	H. J. Brodie	

* This column refers to the number of plants measured or scored in securing the averages used in constructing the polygons (table 2). Where two figures are given, the first indicates the number of plants used to score the first three characters, and the second the number used for the remaining four characters.

3. The *"leaf index"* of some populations of ssp. *fallax* is the same as in populations of ssp. *petiolaris* but again the lowest value .32 is found in the Southwest in *Arizona 41*, while the highest index, .58, is found in *Texas 27*. Plants from Missouri, Oklahoma, and Texas in general have high values for this character. The

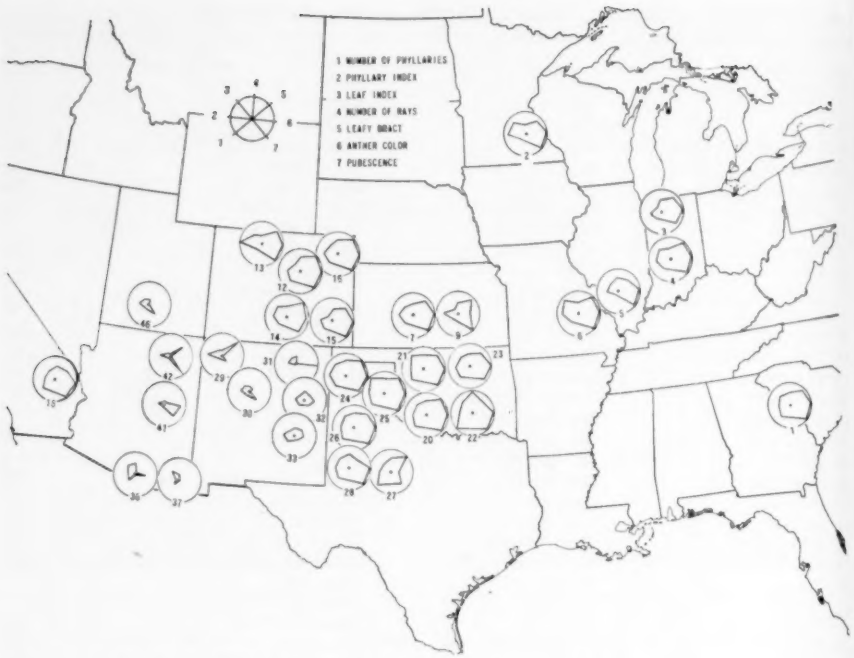


FIG. 2. Polygonal graphs illustrating variation from population to population in *Helianthus petiolaris*, based on mean values of first four characters and percentages of last three characters. Sources of plants and numbers of plants employed in constructing graphs given in table 1. For further information see text.

leaves in the southwestern populations tend to be larger than those from other areas.

4. The *number of rays* ranges from 13 in *Arizona 42* to 25 in *Oklahoma 22*, and in general a low ray number is characteristic of ssp. *fallax*. Ray color was also scored, and all populations had orange-yellow rays except the two from southern Arizona, which were characterized by paler colored rays.

5. The presence or absence of a *leafy bract* conspicuously larger than the phyllaries shows some variation from head to head on a single plant, but if single heads are selected at random from each plant of a population and then scored for this character, conspicuous differences are seen between the populations from the Southwest and other regions. In most of the populations ouside of the Southwest 100% of the heads lacked the bract, whereas with the exception of *New Mexico 29* most of the plants referable to ssp. *fallax* possessed the bract.

6. Two main *colors of anthers* could be distinguished. Southwestern populations with the exception of *New Mexico 31* had all or a majority of the plants with reddish anthers, whereas nearly all plants of populations from other areas had purple anthers.

7. *Stem pubescence*. The peduncle or upper portion of the stem of *H. petiolaris* is moderately or densely covered with short (.1–.3 mm) slender, appressed hairs. In addition, some plants have a few hairs that are longer, thicker, and spreading. Herbarium specimens from the garden cultures were scanned under a binocular scope

for type of pubescence, and in figure 2, the results are expressed in percentage of plants lacking the large trichomes. As can be seen from the figure, most of the populations of ssp. *fallax* with the exception of *Arizona 42* are characterized by the presence of the large trichomes. *Arizona 42* was also exceptional in that it also showed few hairs of the smaller type.

The polygons show that there is considerable variation in *H. petiolaris*, although it is not as extreme as that in *H. annuus* (Heiser, 1954). There are no evident clines, however, such as were apparent in *H. debilis* ssp. *debilis* (Heiser, 1956). The greatest inter-populational variation is in the Southwest, and this may be explained in part by more effective isolation and perhaps by hybridization with the other subspecies to the north and east. From a taxonomic standpoint the southwestern populations, *H. petiolaris* ssp. *fallax*, appear sufficiently distinct to warrant taxonomic recognition. The California population, on the other hand, clearly belongs with the more eastern *H. petiolaris* ssp. *petiolaris*. It will be shown that this relationship also is borne out by the crossing behavior, so it is logical to assume that California populations represent introduction from somewhere other than the Southwest. The Minnesota population, which will be shown to differ from all the other groups in crossing behavior, does not differ greatly morphologically from other populations of *H. petiolaris* ssp. *petiolaris*. The plants from Minnesota come into bloom earlier than the other races and are slightly shorter but this hardly seems sufficient grounds for giving them taxonomic recognition.

Hybridizations and Cytology

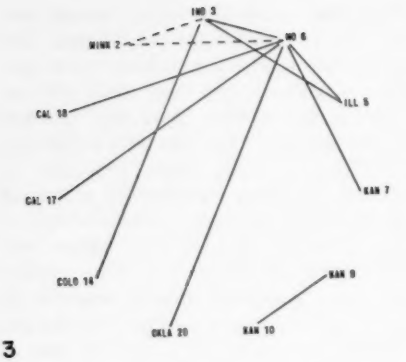
Methods.—Reciprocal crosses were made between garden-grown populations from different sources, although the same individuals were not necessarily used as both male and female parents. Nearly all pollinations were successful, usually resulting in abundant seed set, except in the year 1958, when unusually heavy rainfall was probably responsible for the failures. The F_1 hybrids may be grouped into three general categories: (1) those with 90% or better pollen fertility, good seed set, and normal meiosis; (2) those with pollen fertility around 50%, slight reduction in seed set, and some irregularities at meiosis (generally 15 pairs and a chain or ring of four chromosomes); and (3) those with considerable reduction in pollen fertility (15–50%) somewhat greater reduction in seed set, and greater meiotic irregularities (generally 14 pairs and a chain or ring of six chromosomes).

Reciprocal crosses also were made within each geographic sample, and these, with the exception of certain Texas populations, gave F_1 plants with high fertility (90–100% stainable pollen) and with 17 II's at meiosis.

Aceto-carmine smears of pollen mother cells were made for most of the hybrids and 10–25 cells were analyzed at diakinesis from two hybrid plants of each combination. Slides of several of the hybrids have been made permanent. Pollen was stained with cotton blue in lactophenol, and those grains taking a dark blue stain were considered good. At least five plants were analyzed for each population, and two hundred grains were analyzed for each plant. Seed set was estimated by breaking open nearly mature heads and examining the number of darkening achenes.

The first crosses between races of *H. petiolaris* were made in 1949 and 1950. No plants of this species were grown during the period from 1951 through 1953, and some of the earlier races were lost. Therefore, crosses in all possible combinations have not been made, but sufficient information has now accumulated to indicate a general pattern in regard to fertility of the F_1 hybrids.

Results.—The results of crosses between certain members of *H. petiolaris* ssp. *petiolaris* are given in figure 3, and it can be seen that plants from Indiana, Illinois, Missouri, Kansas, Colorado, California and



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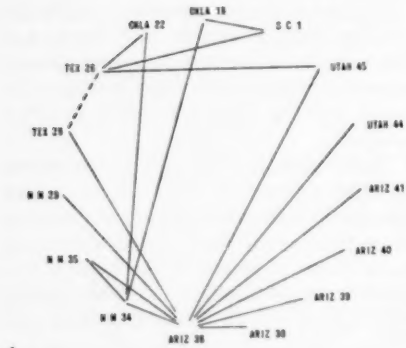

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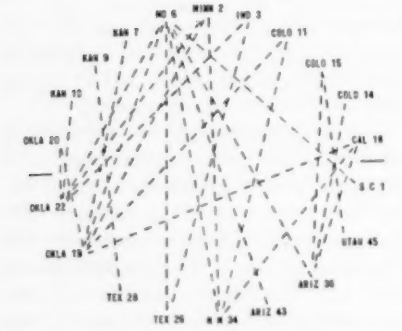

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FIG. 3–5. Crossing polygons of different geographical samples of *H. petiolaris*. FIG. 3. Group A and Minnesota. FIG. 4. Group B. FIG. 5. Crosses between members of different groups. A solid line indicates that the F_1 hybrid was fertile; a dotted line that the F_1 showed reduced fertility. All hybrids were secured reciprocally with the exception of the following: Okla. 20 x Mo. 6, Ill. 5 × Ind. 3 (fig. 3); Ariz. 36 × Texas 26, Ariz. 36 × Texas 28, Okla. 19 × NM. 34 (fig. 4); Texas 26 × Ind. 3, Okla. 22 × Ind. 3 (fig. 5), in which the hybrid was secured only with the first listed plant as the female parent.

one of the populations from Oklahoma all gave fertile hybrids, which regularly showed 17 bivalents at diakinesis. This assemblage of the species will be referred to as Group A. It cannot be certain that all Kansas samples belong to this assemblage, since the appropriate crosses were not made.

The Minnesota plants gave hybrids with fertilities of 35 to 90% when crossed with two other (fig. 3) samples. Examination of meiosis in these hybrids revealed that about half of the cells in the hybrids had circles or chains of 4 chromosomes and 15 pairs, and the remainder had 17 pairs.

Figure 4 gives the results of the crosses of certain other samples (Group B), including some referable to *H. petiolaris* ssp. *petiolaris* (Texas, Oklahoma, and possibly South Carolina) and others to *H. petiolaris* ssp. *fallax*. The majority of the F_1 plants were fertile (90% or more pollen stainable) and showed 17 II's at meiosis. One exception was *Utah 44 × Arizona 36*. With Arizona as the female parent fertile hybrids were secured, but the reciprocal combination gave weak, partially sterile plants. The latter, however, had normal meiosis so it does not appear that the sterility was due to structural differences of the chromosomes. The crosses of South Carolina with both Oklahoma and Texas produced some hybrids with pollen fertility as low as 70% but other hybrids of the same crosses gave pollen fertilities of 90% or better.

Crosses of members of Group A and Minnesota with members of the B Group are given in figure 5. All the hybrids showed reduced pollen fertility, ranging from 8 to 50% with a mode at 34%. One-half to approximately two-thirds of the achenes of each head were filled. Meiosis was examined in all hybrid combinations, except *5 × 55* and *15 × 61*. In all plants the majority of cells showed 14 pairs and

either a circle or chain of 6, some cells showed 15 pairs and 1 IV, and in a very few cells 17 II's were observed.

The only unexpected situation encountered involved the two Texas populations. In figure 5 it is shown that *Texas 26* gave hybrids with reduced fertility when crossed with two members of the A Group and in figure 4 it is shown that both *Texas 26* and *28* gave fertile hybrids with other members of the B Group. Therefore, it was expected that *26* × *28* would give fertile hybrids when intercrossed, but such was not the case. The hybrids between these two populations show reduced fertility and 14 pairs and a chain of 6 were observed at diakinesis. The same individual plants in these populations were not used for all the crosses, and the only explanation that can be offered at present is that translocations may be floating in the Texas populations. This is also suggested by the fact that intercrosses within one population gave offspring with reduced fertility. The pollen fertilities were checked on the original plants used in the crosses and all were found to be 90% or higher. Out of 15 plants collected from *Texas 43*, thirteen had 90% or better pollen fertility and two gave counts of 54% and 69%. It is hoped that additional work can be done with the Texas populations.

Discussion of Hybrids within *H. petiolaris*

On the basis of the fertility of hybrids all the samples of *H. petiolaris* ssp. *fallax* belong to one crossing group (B). Certain representatives of *H. petiolaris* ssp. *petiolaris* (Oklahoma, Texas, and South Carolina) belong to this same crossing group and others (Indiana, Illinois, Missouri, Kansas, Colorado, California, and part of Oklahoma) constitute a second distinct group (A) with the exception of Minnesota which falls into a group by itself. Whether the Minnesota type is widespread or limited to the one population is, of course, not yet known.

The map (fig. 1) shows that the number of samples is fairly adequate except for the northern Great Plains. Plants from this area show minor morphological differences from *H. petiolaris* ssp. *petiolaris*, but not enough to deserve taxonomic recognition. Which assemblage they belong to, or if they constitute still a separate group, is not known. As mentioned previously, plants from Wyoming and Canada have been grown in the garden but did poorly so that crosses with other samples were impossible.

It is of interest that the chromosomally homologous assemblages do not coincide with the morphological groups, although as Clausen (1951) points out, cytological and morphological differentiation do not always proceed hand in hand. From a cytological standpoint certain of the populations from Oklahoma and Texas are identical with *H. petiolaris* ssp. *fallax* although their genic content is more similar to that of plants of *H. petiolaris* ssp. *petiolaris*. Two possible explanations may be advanced: (1) The chromosome end arrangement of Group B is the more primitive, and in this group morphological differentiation took place, perhaps promoted by geographical isolation which is no longer present, to give ssp. *fallax* in the Southwest and ssp. *petiolaris* in Oklahoma and Texas. Subsequently translocations developed within ssp. *petiolaris* giving rise to Group A; or (2) the ancestral arrangement is that of Group A. Reciprocal translocations occurred giving rise to Group B without morphological differentiation, and then presumably through geographical isolation ssp. *fallax* arose in the Southwest.

On the basis of the chromosome configurations observed in the artificial hybrids it is possible to speculate upon the chromosome end arrangements present in the various groups. Let us assume the standard arrangement for Group B to be 1:2 3:4 5:6 7:8, etc. This differs from Group A by two translocations involving three chromosomes to give the 14 pairs and a circle or chain of six chromosomes usually observed in hybrids between Group A and B, so that the end arrangement of Group

A would be 1:5 2:3 4:6 7:8, etc. Since Minnesota differs by one translocation from Group A (giving hybrids with 15 II's and a IV) and by two translocations in three chromosomes from Group B, its end arrangement could be 1:3 2:5 4:6 7:8, etc. These postulated end arrangements are probably oversimplifications since additional structural changes of a cryptic nature may be involved, but they do provide a working hypothesis for additional discussion.

Hybrids of H. petiolaris *with other species.*—Attempts at crossing *H. petiolaris* with the perennial species, *H. carnosus* Small, *H. decapetalus* L., *H. mollis* Lam., *H. pumilus* Nutt., and *H. silphioides* Nutt. have been unsuccessful, but hybirds have been secured with the annual taxa, *H. annuus* L., *H. argophyllus* T. and G., *H. Bolanderi* A. Gray, *H. debilis* Nutt., and *H. neglectus* Heiser and *H. canus*. In all of these the original hybridizations gave many seeds and the F_1's were vigorous, although showing considerable impairment of fertility. The only annual with which hybrids were attempted and not secured is *H. agrestis* Pollard, a rather anomalous species from Florida which as yet has not been successfully crossed with any other species.

(1) Hybrids with *H. annuus*. Natural and artificial hybrids of *H. annuus* and *H. petiolaris* have been described previously (Heiser, 1947). Since that time several additional natural hybrids have been found (see fig. 1), and five additional artificial hybrid combinations have been made. The hybrids usually have pollen fertility of less than 10% and seed set of 1% or less. Although in the earlier report it was suggested that these two species differ by an inversion, no additional evidence for this has been secured, and it seems possible that univalents were interpreted as fragments in the earlier work. The interpretation of the configurations at meiosis is not as simple as for the hybrids previously discussed because of the greater number of irregularities. The number of pairs ranges from 7 to 15 with the remainder of the chromosomes associated in rings or chains. Hybrids of *H. annuus* with members of Group A of *H. petiolaris* generally show poorer pairing than hybrids with Group B, although the pollen fertilities of the latter hybrids run somewhat lower than those of the former. On the basis of stem pubescence, phyllary length, and leaf color *H. annuus* appears more closely related to ssp. *fallax* than to ssp. *petiolaris*. The possibility that ssp. *fallax* owes its origin to introgression of *H. annuus* into ssp. *petiolaris* deserves critical study. It is certainly evident that natural hybridization between the two species is common. The amount of introgression that may result from this hybridization is very difficult to assess, although as pointed out earlier (Heiser, 1947) the variability of both species has probably been enriched by the process in spite of the partial sterility.

(2) Hybrids with *H. argophyllus*. Crosses of *H. argophyllus*, a species closely related to *H. annuus* and restricted to eastern Texas, have been made with one representative of both Groups A and B of *H. petiolaris*. The pollen fertility in the hybrids ranged from 15–28%, and the most frequent configuration at diakinesis was thirteen pairs and two circles of four, although some cells showed three circles of four chromosomes. Seed set was below 10%.

(3) Hybrids with *H. Bolanderi*. Two representatives of Group B and one of Group A of *H. petiolaris* have been crossed with *H. Bolanderi*, a species of California and Oregon. All three sets of hybrids had low fertility, with good pollen ranging from 1–14% and seed set from 2 to 6%. Meiosis in hybrids with Group B revealed 10 to 14 pairs with the remainder of the chromosomes associated in chains.

(4) Hybrids with *H. debilis*. *Helianthus debilis* comprises eight subspecies which fall into two crossing groups[3] (Heiser,

[3] The two groups might with some justification be regarded as separate species, but for convenience the classification adopted in the earlier paper will be followed here.

1956): *H. debilis* ssp. *debilis* of Florida and *H. debilis* ssp. *cucumerifolius* of east central Texas belong to one group, and *H. debilis* ssp. *praecox* of eastern Texas and *H. debilis* ssp. *hirtus*, Dimmit Co., belong to the other. The first two subspecies give fertile F_1 hybrids with normal chromosome pairing when intercrossed as do the last two, but crosses between members of the first group and those of the second produce F_1 hybrids with reduced fertility. These latter hybrids showed 15 II's and a chain of IV in a majority of the cells, although some cells were observed in which 13 II's and two chains of IV occurred, so that apparently two translocations are involved although only one is usually expressed.

The following reciprocal crosses of *H. debilis* ssp. *cucumerifolius* or ssp. *debilis* were made with *H. petiolaris*:

X Arizona 61	(Group B)	range of pollen fertility	11–27%
X South Carolina	(Group B)	range of pollen fertility	16–26%
X Oklahoma 16	(Group B)	range of pollen fertility	9–28%
X Missouri 5	(Group A)	range of pollen fertility	10–23%
X Minnesota 2	(MINN.)	range of pollen fertility	20–40%

In all these the most frequently observed configuration was 14 pairs and an association of 6, although in all except the hybrids with South Carolina some cells were observed with fewer than 14 pairs but the exact configuration could not be determined.

Crosses of either *H. debilis* ssp. *praecox* or *hirtus* with *H. petiolaris* were as follows:

X New Mexico 55	(Group B)	range of pollen fertility	44–73%
X South Carolina 1	(Group B)	range of pollen fertility	59–70%
X Oklahoma 16	(Group B)	range of pollen fertility	44–73%
X Missouri 5	(Group A)	range of pollen fertility	14–17%
X Minnesota 2	(MINN.)	range of pollen fertility	34–43%

All the hybrids showed 15 II and a chain or circle of IV at diakinesis.

Morphologicaly *H. debilis* ssp. *praecox* and *hirtus* are somewhat intermediate between *H. petiolaris* on the one hand, and *H. debilis* ssp. *debilis* and *cucumerifolius* on the other, and the close relationship of the former subspecies to *H. petiolaris* is clearly indicated in the results of the hybridizations. In fact, the fertility of hybrids between *H. petiolaris* and *H. debilis* ssp. *hirtus* and ssp. *praecox* is generally higher than those in hybrids between the two groups of *H. debilis*.

Previously, the following tentative end arrangements for *H. petiolaris* were postulated:

Group	B	1:2	3:4	5:6	7:8, etc.
Group	A	1:5	2:3	4:6	7:8, etc.
Group	MINN.	1:3	2:5	4:6	7:8, etc.

Therefore, on the basis of the above crosses the arrangement of 1:2 3:5 4:6 could be postulated for *H. debilis* ssp. *praecox* and *hirtus*, which would give 15 pairs and a chain of four chromosomes with all crossing groups of *H. petiolaris*.

Since these subspecies of *H. debilis* generally give 15 pairs and a chain of four chromosomes with the subspecies *debilis* and *cucumerifolius* and they in turn give 14 pairs and a chain of six with *H. petiolaris*, the end arrangement of the first three chromosomes of *H. debilis* ssp. *debilis* and *cucumerifolius* may be 1:6 3:5 2:4. As pointed out above, however, in some crosses between the two crossing groups of *H. debilis* thirteen pairs and two associations of four chromosomes were seen, and in most of the hybrids of *H. petiolaris* with *H. debilis* ssp. *debilis* or *cucumerifolius* less than fourteen pairs were observed; therefore, it seems likely that these two subspecies of *H. debilis* differ by at least one other translocation from the other groups.

(5) Hybrids with *H. neglectus*. Crosses of *H. neglectus*, a species very similar to *H. petiolaris*, and occupying a very limited area in western Texas (fig. 1) have been discussed elsewhere (Heiser, 1958). With both Groups A and B of *H. petiolaris* it gave hybrids showing fifteen pairs and a chain or ring of four chromosomes, or rarely thirteen pairs and two associations of four chromosomes. Only two crosses have been made between *H. debilis* and *H. neglectus*. With *H. debilis* ssp. *praecox* the hybrids showed thirteen to fifteen pairs

and one to two chains. In hybrids with *H. debilis* ssp. *cucumerifolius* the number of pairs ranged from 11 to 15. It is not possible to work out an end arrangement for *H. neglectus* that will agree with the cytological observations and the arrangements previously postulated for the other species.

(6) Hybrids with *H. canus*. This taxon, although previously considered a variety of *H. petiolaris*, has been difficult to cross with other members of this complex. With Group A of *H. petiolaris* as the female parent a single hybrid was secured which showed 36% good pollen. No suitable stages were obtained for analysis of meiosis. With Group B as the male parent three hybrids were secured in one cross and a single hybrid in the second cross. The pollen fertilities of these hybrids ranged from 5 to 18%, and from 10 to 13 pairs were observed at meiosis with the remaining chromosomes associated in chains. A single hybrid was secured with *H. neglectus* as the female parent and it had a pollen fertility of 9% and showed 11 to 12 bivalents and chains. With *H. debilis* ssp. *debilis*, on the other hand, reciprocal crosses were successful but only one seedling germinated for each combination. One hybrid gave 21% good pollen and the second 23%. Both hybrids regularly showed 15 pairs and a circle or chain of four chromosomes.

These results are somewhat difficult to explain. Morphologically *H. canus* appears to be most closely related to *H. petiolaris* ssp. *fallax* and their ranges overlap to some extent, yet hybrids between the two have quite low pollen fertilities and there is indication of considerable structural difference in their chromosomes. On the other hand, hybrids with *H. debilis*, while showing rather low pollen fertilities, were found to differ by only a single translocation. It is, of course, possible that each has descended from ancestors with quite different chromosome arrangements, and that by chance translocations have accrued to give them 15 chromosomes with the same end arrangement. It is also quite possible that both are quite close to the "primitive" structural arrangement and that one or both have migrated from the center of origin with chromosomal differentiation proceeding more rapidly in the taxa (*H. petiolaris* and *H. neglectus*) which are now occupying the intermediate part of the range. On the basis of the little evidence available at present one cannot choose between these two hypotheses.

Discussion

Comparison of the crossing behavior and chromosome pairing in hybrids within the three annual sunflowers, *H. annuus*, *H. debilis*, and *H. petiolaris*, shows that these have quite different patterns. *Helianthus annuus*, which has attained the widest distribution and is the most "weedy" of the three, is perhaps the most variable morphologically, although it is the species in which it is most difficult to recognize clear-cut subspecific taxa. Fewer crosses have been made within this species than within the other two, but all of those which have been obtained, using cultivated as well as wild material, have given fertile F_1's and no indication has been obtained of structural differences in the chromosomes (Heiser, 1954). In *H. debilis*, which occupies the most limited area of the three, well defined subspecies may be recognized, perhaps in part because geographical isolation has been more effective. Two structural races are known within this species and it is generally possible on the basis of morphology to predict the structural arrangement of a given plant.

In *H. petiolaris*, on the other hand, subspecies may be recognized which are better defined than in *H. annuus* but less clearly so than in *H. debilis*. This species also differs from the others in that three chromosomal arrangements are known for one subspecies. From the study of *H. petiolaris* it is also clear that morphological differentiation is not always correlated with structural difference of the chromosomes.

Translocations may be important in initiating reproductive isolation, and it is also

known that certain structural arrangements may confer a selective advantage (Stebbins, 1958). In a letter (April 7, 1958) Stebbins points out that on mathematical grounds it is highly unlikely that random translocation alone can account for the end arrangements postulated for *H. debilis* and *H. petiolaris*, and he suggests two possibilities. One, that the different types originated at one time from a hybrid complex segregating for translocations involving the three chromosome pairs in question. This, however, he feels would be highly improbable in view of the fact that five taxa are involved, some rather far removed from any possible center of origin. The second possibility is that these three chromosome pairs contain gene combinations with a particularly high selective value, and that selection pressure has established translocations involving these three chromosome pairs much more frequently than translocations involving other chromosomes.

Although in some groups reciprocal translocations have proven a useful tool in attempting to trace phylogenies (Cleland, 1950), in *Helianthus* they seem to have limited value in this regard. The variability of chromosome pairing in many of the hybrids might indicate that small segments of chromosomes have been translocated rather than whole arms, which makes it difficult to arrive at definite chromosome end arrangements for certain of the taxa. Moreover, there is no reason to assume that any given end arrangement is the ancestral type.

In view of these various objections it may be presumptious to postulate even a hypothetical phylogeny. However, some discussion on the subject may be in order, and it may serve as a target for criticism. Morphologically, there is no evidence for regarding any of these sunflowers as more primitive than others. There is some evidence, however, that *Helianthus* had its origin from the genus *Viguiera*, or came from the same gene pool as that genus (Heiser and Smith, 1955; Heiser, 1957). *Viguiera*, with the exception of one anomalous species in the southeastern United States, reaches its northernmost limits in the Southwest. For that reason one might be inclined to think of the Southwest or northern Mexico as the starting point of the genus *Helianthus*. From this southwestern center a species may have spread widely and then become broken up into the taxa which have been discussed here. Or possibly there was a stepwise change into new species, following migration and isolation, in a manner somewhat as shown in figure 6.

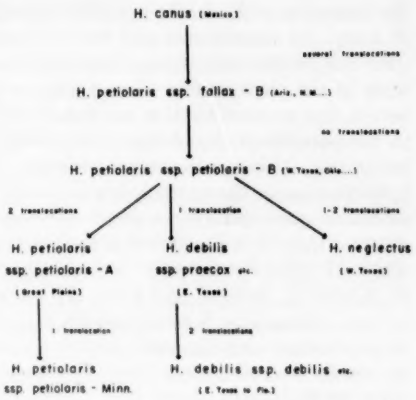

FIG. 6. Hypothetical "family tree" of *H. petiolaris* and close relatives.

Helianthus canus is regarded as the starting point solely from the standpoint that geographically and morphologically it stands nearest to *Viguiera*. The northernmost race of *H. petiolaris* from Minnesota and the southeastern races of *H. debilis* are the farthest removed from the ancestral type, since it would seem reasonable that these species or their ancestors moved into the glaciated northern areas and the recently emerged southeastern area from some outside center, but whether or not the genus is old enough for these geological factors to have any effect is not known. This phylogenetic scheme can be defended on both morphological and cytological grounds, but it would be possible to erect other schemes that are almost as logical. For example, an original dichotomy might have resulted in

the formation of *H. debilis* ssp. *debilis* from *H. canus* (or the reverse) and they in turn gave rise to the other types. One possible fault of the phylogenetic scheme proposed here is that no consideration has been given to the possible origin of taxa through hybridization. Although the rather widespread hybridization in the genus today is largely associated with disturbance of the environment by man, it is possible that hybridization took place in the past. For example, *H. debilis* ssp. *praecox* and *hirtus* are more or less intermediate between certain forms of *H. petiolaris* and the other subspecies of *H. debilis* and a hybrid origin for them might be postulated.

Where *H. annuus*, *H. argophyllus*, and *H. Bolanderi* should be placed in relation to the other species is somewhat obscure at present. Although these three species obviously are closely related to the other taxa under consideration here, they do not show as close a relationship as do these other taxa among themselves.

Summary

From a study of seven morphological characters of 33 garden-grown populations of *H. petiolaris* from different geographical areas it is shown that the two subspecies, *H. petiolaris* ssp. *petiolaris* and *H. petiolaris* ssp. *fallax* have conspiciously different variation patterns. Artificial hybrids were made between plants from different populations. From the study of the fertility of F_1 hybrids three groups may be recognized: Group A from Indiana, Illinois, Missouri, Kansas, Colorado, California, and in part, Oklahoma, belonging to ssp. *petiolaris*; Group B from South Carolina, Oklahoma, in part, Texas, Arizona, New Mexico, and Utah, belonging to both ssp. *petiolaris* and ssp. *fallax*; and Group MINN., based on a single sample from Minnesota identified as ssp. *petiolaris*. Crosses between members of populations within either Group A or B give fertile hybrids, with one exception from Texas, whereas crosses between members from the different groups give F_1's with reduced fertility. Studies of diakinesis reveal that members of Group A differ from members of Group B by two translocations and from MINN. by one translocation, and that MINN. differs from members of Group B by two translocations. Theoretical chromosome end arrangements are postulated based on these results. The morphological races recognized in *H. petiolaris* do not coincide with the "cytogenetic" groups in contrast to the situation found in *H. debilis*.

Artificial F_1 hybrids of *H. petiolaris* with *H. annuus*, *H. argophyllus*, *H. Bolanderi*, *H. debilis*, *H. neglectus*, and *H. canus* are briefly discussed. All the hybrids, with a few exceptions, show rather highly reduced fertility. Tentative chromosome end arrangements are postulated for *H. debilis*. A hypothetical "family tree" of *H. petiolaris* and its close relatives is presented in which it is assumed that *H. canus* is the "ancestral" species.

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NOTES AND COMMENTS

EVIDENCE FOR GENETIC DRIFT IN INDIAN RATS (*RATTUS RATTUS* L.)

HANS GRÜNEBERG

Experimental Genetics Research Unit (Medical Research Council), University College, London

SKELETAL VARIATION IN MICE

Inbred strains of laboratory mice (*Mus musculus* L.) differ greatly from each other in a multitude of minor skeletal variants (Grüneberg, 1950, 1952; Truslove, 1952, 1954; Searle, 1954a, b; Deol, 1955). Some of these variants are strict all-or-none characters, like the presence or absence of fusions between cervical vertebrae; others can be classified into normals and abnormals, but the abnormality, when present, may itself be a graded character (like absence of third molars which may involve from one to four such teeth). Finally, there are metrical characters like the size of the spinous process of the second thoracic vertebra which, for ease of classification, can be grouped into a number of arbitrary size classes. Each of more than 30 such variants so far described occurs in a given inbred strain with a constant frequency. The extensive differences between inbred strains kept under constant environmental conditions show that these minor variations are largely under genetic control. However, the genetic basis is complex and multifactorial, and

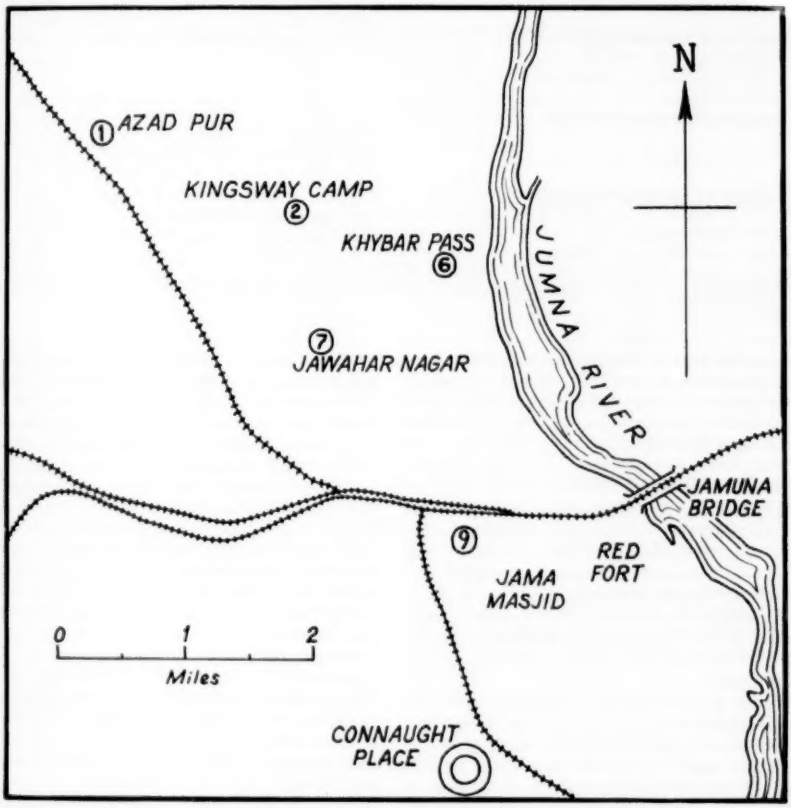

FIG. 1. Sketch map of the northern part of Delhi. The origin of populations Delhi-1, 2, 6, 7, and 9 is indicated.

TABLE 1. *Frequency of 8 skeletal variants in 5 populations of Delhi rats*

Variant		D-1	D-2	D-6	D-7	D-9	$\chi^2_{(4)}$	P
1. Abnormal metoptic roots of presphenoid	– –	25	19	26	28	26	11.806	0.02
	–+	1	3	0	2	2		
	++	4	8	4	0	2		
2. Processus pterygoideus	– –	17	20	18	20	24	5.048	0.28
	–+	7	5	7	4	5		
	++	6	5	5	6	1		
3. Accessory processus petrosus	– –	22	17	15	23	19	4.817	0.30
	–+	3	2	8	1	5		
	++	5	11	7	6	6		
4. Foramen hypoglossi double	– –	26	28	11	18	24	31.921	2×10^{-6}
	–+	3	1	11	11	5		
	++	1	1	8	1	1		
5. Double arch foramina in C 3	– –	28	25	24	21	28	6.740	0.15
	–+	2	3	4	5	1		
	++	0	2	2	3	1		
6. Small arch foramina in C 4 or C 5	– –	28	19	26	21	24	9.561	0.05
	–+	2	9	2	7	3		
	++	0	3	2	2	3		
7. Number of thoracic vertebrae	13	18*	22	26	18	23	6.710	0.15
	13½	6	2	1	2	2		
	14	6	6	3	10	5		
8. Number of presacral vertebrae	26	27*	27	29	27	29	.966	0.92
	26½	1	0	0	2	0		
	27	2	3	1	1	1		

* Includes one specimen with 12 thoracics in which one such vertebra has probably been accidentally lost.

evidence has been produced that many or most of these discontinuous variables are, in fact, quasi-continuous, i.e., satellite characters of metrical variants which in most cases remain to be identified (Grüneberg, 1951, 1952).

POPULATION DIFFERENCES IN MICE

Early in these investigations, it was discovered (Weber, 1950) that the same kind of skeletal variation occurs in wild mice, and that a number of wild populations collected in England, Scotland, and Switzerland differed strikingly from each other in the incidence of some of these variants. More recently, Deol (1958) recorded similar findings for four wild populations of mice from the eastern United States.

Whereas it is obvious that these interpopulation differences are mainly genetic in nature, the data at hand do not give any information as to the mechanism which has brought these differences into being. Most of the skeletal variants studied are, or appear to be, trivial in relation to the biological fitness of the individual; however, they seem to be satellite characters of metrical moieties which may well have selective significance. As most of the populations came from widely separated localities with different climatic and other environmental conditions, the interpopulation differences might be the result of selection in adaptation to different environments. The alternative hypothesis is that the variants studied came to differ in frequency in different populations as the result of genetic drift; if so, the various localities were first invaded by small numbers of mice which subsequently multiplied and remained more or less isolated from other populations. Under this (stochastic) interpretation, the interpopulation differences would not be adaptive in nature.

SKELETAL VARIATION IN THE RAT

In October and November, 1959, I had the opportunity to collect the skeletons of 150 adult or nearly adult rats (*Rattus rattus* L.) in Delhi (India); they were prepared by the papain maceration technique and came, in batches of 30, from five small grain shops as shown in figure 1. In each case, the shops were terraced and of one story only; during the day, the front of the shops is widely open, by night

they are closed by shutters. Traps were set in the grain shops themselves and, in most instances, also in the adjacent shops on either side.

Skeletal variation in the rat is essentially similar to that in the mouse, and the same names have been used for identical or closely similar phenotypes. Many variants peculiar to a single inbred strain of mice have not (? yet) been encountered in the rat. Other differences seem to arise from anatomical causes. For instance, in the mouse where the two frontals normally remain separate throughout life, there exist variants like interfrontal, parted frontals, and frontal fontanelle which have not been encountered in the rat whose frontals tend to fuse across the midline in adults. Similarly, the absence of dysymphysis of Th 1 and of Th 10 in the rat is probably not due to an accident of sampling, and the spinous process of Th 2 which is proportionately much bigger in the rat seems to be less variable in size than in the mouse.

Despite these differences in detail, the essential similarity of skeletal variation in the two species is obvious and unmistakable, and there can be little doubt that the underlying genetic basis is similar in rat and mouse.

Population Differences in the Rat

In table 1, eight of the commoner variants have been tabulated by population: $- -$ means that the variant is present on neither side, $- +$ that it is present on one, and $+ +$ that it is present on both sides. In the case of the number of thoracic vertebrae, 13½ means that vertebra 21 had a transverse process on one side but not on the other (where it presumably carried a rib). In the case of the number of presacral vertebrae, 26½ means that the os innominatum articulates on one side with vertebra 27 and on the other with vertebra 28. With the possible exception of No. 3, all variants also occur in the mouse, but Nos. 5 and 6 have not been worked out in detail in that animal. In the last column but one, a heterogeneity χ^2 (for four degrees of freedom) is given; it has been calculated according to an adaptation of Yates's (1948) method, which I owe to Dr. C. A. B. Smith.

Turning now to the actual data, it is obvious that the populations differ highly significantly as regards the incidence of "foramen hypoglossi double" (No. 4), a character whose classification is completely objective. The offending population is obviously Delhi-6; if that is omitted, the remaining four populations probably are still not homogeneous ($\chi^2_{(3)} = 7.598$; $P = 0.055$); the offending population is now Delhi-7; as this differs significantly from Delhi-6 ($\chi^2_{(1)} = 6.262$; $P = 0.012$), it seems that there are actually three different frequencies of this variant in our populations. In addition, two other variants (Nos. 1 and 6) show heterogeneity between populations at a lower level of significance ($P = 0.02$ and 0.05 respectively). There is thus no doubt that the five populations cannot be regarded as samples of a single superpopulation, but that they differ significantly from each other in their phenotype and hence in their genetic constitution.

Table 2. *Some rarer skeletal variants in Delhi rats*

Variant	D-1	D-2	D-6	D-7	D-9
Preoptic sutures in presphenoid	0	3	0	1	0
Absence of upper third molars	0	0	0	1	0
Fusions between cervical vertebrae*	1	2	0	1	0
Foramina transversaria imperfecta**	0	1	1	1	1
Dystopia cranialis tuberculi ant.***	0	0	0	0	1
Dystopia caudalis tuberculi ant.***	0	0	0	1	0

* C2 + 3 (twice), C3 + 4 (once), C4 + 5 (once).
** C6 (thrice right, once left).
*** C6.

For sake of completeness, some of the rarer variants are given in table 2. They supply no information concerning interpopulation differences.

Discussion and Summary

The rat populations described in this paper differ from the mouse populations of Weber (1950) and Deol (1958) in that they all come from the same city and hence certainly share the same macroclimate. The grain shops in which the animals lived were very similar and provided approximately the same range of food stuffs. Hence it is difficult to maintain that the interpopulation differences encountered have arisen by natural selection in adaptation to different environments. It is much more plausible to assume that the populations each trace back to a small number of foundation animals and hence that they reflect the random genetic differences which are inevitably present under such circumstances. The rat population of Delhi, on this interpretation, is split up into numerous small isolates which differ from each other genetically due to genetic drift rather than to the forces of natural selection.

Whether the same is true of the interpopulation differences in mice must remain an open question. Extensive collections now being carried out by my colleague, Dr. R. J. Berry, will, it is hoped, provide the answer.

Acknowledgments

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TAXONOMY, MOLECULAR BIOLOGY, AND THE PECK ORDER

THEODOSIUS DOBZHANSKY
Department of Zoology, Columbia University, New York City

As in dress, art, and politics, so there are fashions, fads, and fancies in science. At present, molecular biology is at the top, and systematics at the bottom, of the peck order in biology. Two authorities have recently stated bluntly (in *Science* and in the *AIBS Bulletin* respectively) that to be up-to-date a biologist must be a biochemist or a nerve physiologist, and all "natural history" is to be given short shrift. Now, it is true that molecular biology is making spectacular progress; that it is solving some long-standing riddles of nature; and that it is a better-paying proposition than philosophical biology or natural history. And yet organic diversity has and always will have irresistible fascination to many minds; no matter how completely molecular biology tells its story, an immense realm of life processes will remain challenging our ingenuity and inviting study; and these non-molecular aspects of biology happen to be the ones chiefly relevant to understanding man and his place in the universe, to problems of anthropology, sociology, and psychology, in short—to humanism. Much of the work in molecular biology is exciting and thought-provoking, but some of it is intellectually shallow. This also applies, of course, to other fields of biology and of science in general. It is really the assumption that being at the top of the present peck order somehow confers a high intellectual status, together with claims for juicier research grants and academic preferments, that must be disallowed. G. G. Simpson's book* is about taxonomy—near the bottom of the peck order. Some of the problems it deals with are two centuries old. It has no startling new discoveries to report. But it provides a treat for any biologist who derives satisfaction from thought and reflection about life and its manifestations.

Simpson begins by clearing away some terminological log jams. He defines systematics as "the scientific study of the kinds and diversity of organisms and of any and all relationships among them," classification as "the ordering of animals into groups (or sets) on the basis of their relationships, that is, of association by contiguity, similarity, or both," and taxonomy as "the theoretical study of classification, including its bases, principles, and rules." He discusses tolerantly some recent attempts to express taxonomy in terms of symbolic logic, which fail to see any difference between classification of organisms and classification of, say, postage stamps, although he points out that these attempts have not said anything new or suggested any new line of investigation. He comes out squarely in favor of biological classification based on evolutionary descent and relationships, and not solely on morphological similarity or dissimilarity. This is not to deny that "the pre-evolutionary taxonomists, who included brilliant men who did superb work on their own premises, recognized many (far from all!) of these results of evolution without knowing what produced them or what objectively distinguishes them."

The basic difference between the old and the new taxonomy is that the old was based on the idea of morphological types, while the new recognizes populations as the objects to be classified. Typology has a long and honorable tradition; it goes back at least to Plato's eternal and unchanging types and ideas. Implicitly more often than explicitly it has been, and still is, the basis of the thinking of many biologists, including systematists, and among these latter including systematists who pay lip service to evolutionism. Yet, ". . . typological theory or the strictly typological approach should have no part in modern taxonomy. . . . The concept of distinct and static patterns cannot be meaningfully applied to real groups of organisms, which are parts of an evolutionary continuum and which are always highly variable. . . . All populations vary, and the variation is an essential part of their nature and their definition. They do not have single fixed patterns or types." Lest this may seem like flogging a dead horse, witness the recent recrudescence of typological thinking where it would perhaps be least expected—in genetics. The "classical" theory of the genetic population structure regards the existing genotypes mostly as unwelcome deviants from the single optimally fit or "normal" genotype, which the species would have if it were fully adapted to its environment. Consistent exponents of the classical theory envisage mankind as a multitude of persons, ideally all of them carriers of the one "normal" genotype, and hence as similar genetically as identical twins. The fact of the matter is that the habit of typological thinking is deeply ingrained in many minds; it will probably persist no matter how fully its invalidity is demonstrated. It is amusing to speculate that there may be some genes in human populations favoring typological thinking and making their possessors fit in some environments.

Simpson's terse and emphatic statement "The fundamental unit of evolution is the species" should be engraved in every biologist's mind. The definition of species as reproductively isolated groups of sexual and cross-fertilizing populations, proposed and defended for many years by this

* G. G. Simpson. Principles of Animal Taxonomy. XII + 247 pp. Columbia University Press, New York. 1960.

reviewer, by Mayr, and by others, is expounded by Simpson with his accustomed clarity. This definition he describes as "genetical"; I prefer to call it "biological," to emphasize the fact that it is a generalization of findings of many biological disciplines, not of genetics alone. Simpson's answer to the critics of this definition is worth quoting in full: "The definition depends on a criterion, interbreeding, that usually is not and sometimes cannot be observed. That is only a pseudoproblem, as the previous discussion of the relationship between definition and evidence in taxonomy should make clear. The definition does define, and does so in a biologically, evolutionarily meaningful way. The evidence that the definition is met in a given case with a sufficient degree of probability is a different matter. The evidence is usually morphological, but to conclude that one therefore is using or should use a morphological concept of the category (not taxon) species is either a confusion of thought or an unjustified relapse into typology." And further: "There remain numerous doubtful cases where decision depends on the personal judgment of each practitioner of the art of classification. To insist on an absolute objective criterion would be to deny the facts of life, especially the inescapable fact of evolution."

An opinion current in some biological circles is that taxonomy is largely finished business, in which there remains merely the task of expounding the cut-and-dried facts more or less clearly. Anyone holding this opinion should be disabused by Simpson's book. Despite the relatively limited space given to examples and illustration by observational data of the ideas discussed in the book, problems requiring investigation are constantly brought up. As a paleontologist, Simpson is naturally concerned with "paleospecies," species succeeding each other in time within a single lineage. He defines: "An evolutionary species is a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies." That the "genetical" (or as I prefer to call it "biological") species is the core of the "evolutionary" species is made quite clear. Whether the "evolutionary" species makes sense in asexual (uniparental) organisms is a problem widely open. I am inclined to question this, although I agree with Simpson that evolutionary units of some sort do exist in uniparental organisms; what is doubtful is whether these units have much but the name in common with sexual species. How important it is to secure a clear conception of species in paleontology is shown by the situation (which Simpson does not discuss) of the study of human origins. The surfeit of specific and generic names proposed for fossil hominids has obfuscated the fact of profound importance, that most of the evolution of the human stock, since the australopithecine level, has taken place not by branching into many species but by successive transformations within a single specific lineage. Systematists must obviously deal not only with species but also with organic diversity on the infraspecific and supraspecific levels. "Probably the most active and certainly one of the most interesting fields of special study in systematics today is evolutionary investigation of variation within single species or groups of closely related species." Subspecies or race is however the only category which at least sometimes deserves formal recognition in the nomenclature in the shape of a Latin trinomial. Mendelian populations and demes, though of great importance in evolutionary studies, do not enter into classification and should not be named. I must however disagree with Simpson's rating the term "Mendelian population" inconvenient because it is limited to sexually reproducing organisms; to distinguish Mendelian populations from aggregates of uniparental individuals is, indeed, of basic importance both in practical systematics and for clear thinking about evolutionary and taxonomic problems. As to supraspecific taxonomy, "Absolute definitions of higher categories are impossible. One of the unique characteristics of the species is that it can be and is defined without reference to any other category. Definitions of higher categories can only be relative to those of other categories, specifying relative ranks in the hierarchy and set relationships to taxa." This does not, of course, make these higher categories any less necessary in classification, and the last chapter of Simpson's book is a masterful exposition of the science and the art of their use. For, indeed, "There is a science of taxonomy, which adapts the principles of evolution to its special field and adds principles of its own. The principles of taxonomy are applied in classification, which is also an art with canons of taste, of moderation, and of usefulness."

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Lasker, G. W. (ed.). 1960. The Processes of Ongoing Human Evolution. 108 pp. illus. $3.75. Wayne State University Press. A Darwinian centennial symposium addressed to anthropologists and containing six essays dealing with various factors molding current genetic changes in the human species. Climate, culture, blood groups, disease resistance, homeostasis, migration, isolation and radiation are some of the topics considered.

Cold Spring Harbor Symposium on Quantitative Biology. Vol. XXV. Biological Clocks. XIII + 524 pp. illus. $8.00 to individuals; $12.00 to institutions; postage extra. The Biological Laboratory, Cold Spring Harbor, N. Y. A collection of 49 papers and the record of discussions held at the annual symposium of the Long Island Biological Association.

Lasker, Gabriel Ward. 1961. The Evolution of Man. XVI + 239 pp. illus. $3.50. Holt, Rinehart and Winston. N. Y. A brief introduction to physical anthropology, emphasizing the evolutionary and genetic approaches.

ANNOUNCEMENT

The Karl P. Schmidt Fund has modest sums available to assist persons wishing to study at the Chicago Natural History Museum. Grants will be made for study in any of the four fields encompassed by the museum: anthropology (with a natural history orientation), botany, geology (including paleontology), and zoology. An applicant should describe the study proposed in brief terms, state the length of time he wishes to study at the museum and the amount of money needed, and name one reference.

Applications should be mailed to: Chairman, The Karl P. Schmidt Fund, c/o Chicago Natural History Museum, Roosevelt Road and Lake Shore Drive, Chicago 5, Illinois.

REPORT ON THE FIFTEENTH ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF EVOLUTION

Minutes of the Business Meeting

The meeting was called to order at the Biltmore Hotel, New York, N. Y., at 9:15 A.M., December 29, 1960, with President Alfred E. Emerson in the chair. Eighty members were present.

1. The minutes of the last business meeting, held at the Hotel Shoreland, Chicago, Illinois, November 27, 1959, stood approved as published.
2. The Secretary reported on the activities of the Council during the year. In addition to its action on the constitutional amendments, the Council approved the following business by mail ballot: *a*, it rescinded its decision to use the AIBS accounting service and instead allotted $1,000 to be used for clerical assistance by the Treasurer; *b*, it approved a staggered scale of prices for back issues of EVOLUTION; *c*, it established a set of advertising rates for EVOLUTION; and *d*, it approved the office of Managing Editor in order to divide the work of producing EVOLUTION.

   President Emerson appointed D. Dwight Davis, Chicago Natural History Museum, as Managing Editor.
3. The Secretary announced the results of the election for officers and councilors for the Society for 1961.
4. The Editor presented a brief report concerning EVOLUTION.
5. The retiring Treasurer summarized his report for the year ending December 31, 1959, and also an interim report for the first 11 months of 1960. He pointed out that some changes to sustaining membership status, additional memberships, and increased sales of back issues of EVOLUTION, in conjunction with a slightly smaller size of EVOLUTION for 1960, had resulted in a financial situation of a favorable nature. In spite of increased anticipated costs for 1961, he felt that the small increase in dues beginning in 1961 would result in a solvent condition for the Society.
6. The Secretary summarized action taken at the Council meeting held two days before:
   (a) The following Associate Editors were appointed for a three-year term: F. W. Blair, U.S.A. (Vertebrate Zoology); L. L. Cavalli-Sforza, Italy (Human Genetics); Pierre Dansereau, Canada (Botany); G. F. Gause, U.S.S.R. (Microbiology); Hannes Laven, Germany (Cytology); and C. D. Michener, U.S.A. (Entomology).
   (b) The appointment of the Managing Editor was unanimously and enthusiastically confirmed.
   (c) The Council decided to enter into a three-year contract with the Allen Press, Lawrence, Kansas, to publish EVOLUTION.
   (d) Thirteen thousand dollars was approved for the 1961 budget for EVOLUTION.
   (e) The Council approved a motion that proposed constitutional amendments be initiated such that, if carried, the Managing Editor would become a member of the Council.
   (f) The Council transferred to the Endowment Fund the $7,000 now held in bonds and the $1,182.50 received to date as royalties from BEHAVIOR AND EVOLUTION, edited by Roe and Simpson.
   (g) The 1962 meetings were set for Corvallis, Oregon, in conjunction with the AIBS, August 26–30.
7. On behalf of the Council, the Secretary moved: *a*, to add to the Bylaws, Article 1, an additional sentence to read "The dues for life membership shall be twenty (20) times the member rate," and *b*, that this be effective as of January 1, 1961. Motion carried.
8. It was moved that an expression of our appreciation be extended to the officers of the AIBS and the Hotel Biltmore for the facilities and courtesies which have made these meetings possible. Motion carried by acclamation.
9. It was moved that an expression of our appreciation be accorded the retiring Treasurer, Dr. Hampton L. Carson and to Mrs. Carson, for their untiring efforts in the Society's service. Motion carried by acclamation.

The meeting adjourned and President Emerson convened the General Session.

Herbert H. Ross
Secretary

INFORMATION FOR CONTRIBUTORS

Contributions to EVOLUTION may be in the fields of genetics, cytology, plant and animal taxonomy, paleontology, ecology, anthropology, bacteriology, or any other field of biology, provided that the facts and conclusions presented are of primary interest to the student of evolution. Descriptive material shall be included only so far as it is the basis of the evolutionary conclusions. Manuscripts are examined for suitability by members of the Editorial Board or other competent critics.

The length of manuscripts will be limited to twenty printed pages unless an exception is granted by the Editorial Board. Short manuscripts and discussions will be included in a separate section, "Notes and Comments." Half tones and tabular material will have to be kept within economic limits.

Proof should be corrected immediately on receipt, and returned at once with manuscript to the Managing Editor. Authors should leave forwarding directions whenever they move away from the address sent with the manuscript. Ordinarily page proof will not be sent to the author. Costs of changes in proof from the original copy will be charged to the author.

Reprints should be ordered when galley proof is returned. There is usually a delay of several weeks after publication before the author receives the reprints ordered. Illustrations and cuts will be destroyed, unless their return is requested on the reprint order.

Manuscripts should be typewritten in double spacing with ample margins and on only one side of the paper. The Editor should have *one carbon copy in addition* to the original manuscript. The author should keep one carbon copy.

Tables and captions for figures should be typed on separate sheets from the text. The legends for several figures may be placed on a single sheet.

Illustrations, including maps, should be referred to as "figures" wherever possible. Plates are reserved for illustrations that require paper inserts or for collections of small figures. All figures and plates are reduced to a maximum of 4⅝ inches in width and 6⅛ inches in length. Reductions may be stated in the caption but not on the figure.

All references to literature should be collected into a list of "Literature Cited" at the end of the article. See recent numbers of EVOLUTION for accepted style.

Titles should be short, preferably less than seven words. Footnote material should be incorporated in the text whenever possible.

Every article of more than a few pages should have a summary limited to less than four per cent of the length of the paper; it is to be complete in itself, and understandable without reference to the body of the article. The scope of the paper should be stated in an introductory paragraph.

Authors are requested to prepare abstracts of their papers to be returned with the proof to the Managing Editor to be forwarded to Biological Abstracts.

Manuscripts should be addressed to

I. Michael Lerner
Department of Genetics
University of California
Berkeley 4, California

THE SOCIETY FOR THE STUDY OF EVOLUTION

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OBJECTS: The object of the Society for the Study of Evolution, which was founded in March, 1946, is the promotion of the study of organic evolution and the integration of the various fields of science concerned with evolution. The Society endeavors to accomplish this through the publication of the journal and through meetings and working committees.

MEMBERSHIP: Membership is open to all those who are interested in the study of evolution. Members are elected by the Council. There are three classes of membership:

Members—Dues $7.00, including subscription to EVOLUTION
Sustaining Members—Dues $14.00
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All members in good standing receive EVOLUTION.

Applications for membership should be directed to the Secretary, to whom all correspondence regarding membership should be addressed. Institutions may not become members but may subscribe to EVOLUTION. All checks should be made payable to The Society for the Study of Evolution.